David S. Alberts · Lisa M. Hess *Editors*

# Fundamentals of Cancer Prevention

Second Edition

Springer

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## Fundamentals of Cancer Prevention

David S. Alberts · Lisa M. Hess

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## Second Edition

Springer

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David S. Alberts, M.D.

*I would like to dedicate this book to my beautiful wife of 45 years, Heather, who has taught me to "walk the talk" of cancer prevention everyday of my life, and to my magnificent grandchildren, Sammy, Sophie, Sydney, Emma and Tate, who I am certain will carry the banner of health promotion through the century for a much healthier society.*

Lisa M. Hess, Ph.D.

*I would like to dedicate this book to my precious daughter, Rachael, who every moment teaches me the value of health and the beauty of life.*

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# Introduction to Cancer Prevention

# 1

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The goals of cancer prevention are to reduce the incidence, morbidity and mortality due to cancer through the identification and elimination of precancerous lesions (termed intraepithelial neoplasias or IENs) and/or the early detection of minimally invasive cancers. Between 2002 and 2004, the United States (US) saw a 2.1% annual reduction in the total number of cancer deaths (Epsey et al. 2007) . This reduction was primarily due to advances in cancer detection and prevention efforts. For this reduction in cancer death to continue, it is extremely important that we continue to prioritize efforts in cancer prevention and control. Despite the recent reduction in incidence and mortality rates, cancer remains the leading cause of mortality among those under age 85. In the US, cancer accounts for nearly 60,000 more deaths each year than heart disease, the second leading cause of death in this population (Jemal et al. 2007). Cancer is the leading health problem in the US that takes more lives among those under age 85 than any other disease or any accidental causes. Only after age 85 does heart disease surpass cancer deaths.

Cancer is a global term for a variety of diseases that are characterized by uncontrolled cellular growth, enhanced angiogenesis and/or reduced programmed cell death. The site of origin of the disease is used to define general categories of cancer (e.g. breast cancer, skin cancer). Worldwide, the incidence and mortality from cancer has been increasing, despite recent advances in the understanding and treatment of many diseases. This emphasizes the need to define the etiology and molecular basis of cancer and to prevent that cancer from developing. The concept of cancer prevention is changing gradually as we gain a greater understanding of the genetic and molecular basis of carcinogenesis. Certainly, it is understood that the cancer patient is not well one day and the next day diagnosed with cancer. It is estimated that there is an average lag of at least 20 years between the development of the first cancer cell and the onset of end-stage metastatic disease for a broad range of solid tumors. In that there were an estimated 559,650 cancer deaths in the US in 2007 (Jemal et al. 2007), and given the 20+ year lag time, more than 11 million "healthy" Americans harbor ultimately deadly cancers.

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Given the lengthy lag time from the point of the first altered cell to carcinoma, secondary and tertiary prevention strategies represent effective and cost-effective opportunities to dramatically reduce cancer mortality in the next decades. Cancer costs exceeded US$209 billion in 2005 alone (Mackay et al. 2006). These represent economic costs (not considering the psychosocial costs to patients and families) that could be avoided. Chapter 2 discusses the human and economic benefits of cancer prevention in more detail.

Unfortunately, a pervasive problem in the US and many other nations is poor access to health care because of a lack of health insurance (US) and/or lack of services (rural or remote regions and many developing nations). Access to screening programmes and improved health care programmes are essential to prevent cancer. For example, among nations with organized cervical cancer screening programs, the risk of cervical cancer morbidity and mortality has been continuously declining (e.g. Sweden, Finland and France have all seen cervical cancer decrease by greater than 4% per year since the initiation of cervical cancer screening programmes); whereas among nations that lack these programmes, cervical cancer remains a major health risk for all women (e.g. Slovakia and Slovenia have seen annual increases in cervical cancer without these programmes) (Mackay et al. 2006). Similarly, nations that have organized tobacco control policies have shown decreases in youth tobacco use. However, even among nations that have established public health policies, individuals must have access to these programmes for them to be effective. In the US, which has the greatest health care expenditures in the world (US$7.1 trillion per year) and subtantial per capita medical expenses (US$7,000 per person per year), more than 40 million Americans (15% of the total US population) lack even the most basic health insurance coverage, and therefore do not have access to general or preventive health care (Kuttner 2008; IOM 2002). Lack of access to has been demonstrated to result in late cancer diagnosis (e.g. at an advanced stage), cancer treatment delays, and premature mortality (IOM 2002). Even when patients without insurance are diagnosed at the same stage as patients with insurance, they still have a significantly increased risk of death (e.g. patients without insurance have a 30–50% higher rate of death from colorectal or breast cancer than patients with insurance) (IOM 2002).

It is increasingly apparent that virtually all cancers proceed from the first initiated tumor cell (e.g. mutated DNA) to mild, moderate and severe dysplasia, invasive carcinoma (invasion of cells through the basement membrane) and metastatic disease (Fig. 1). A single mutated cell can begin to divide incorrectly and produce additional abnormal cells. Cancer prevention research is working to identify these changes as early as possible to intervene to prevent their progression to cancer. If abnormal cells continue to divide and expand, they can develop into precancerous lesions. These lesions (IENs) can be identified both histologically and by molecular signatures, using a variety of analytical methods (e.g. cDNA microarray) (O'Shaughnessy et al. 2002). They are represented by small, intermediate and advanced adenomatous polyps in the colon, atypical hyperplasia and ductile carcinoma in situ in the breast and simple hyperplasia, atypical hyperplasia and carcinoma in situ in the endometrium. As atypia increases, these dysplasias are believed to develop into cancer, and if left unchecked, has the great potential to metastasize to adjacent and distant organs.

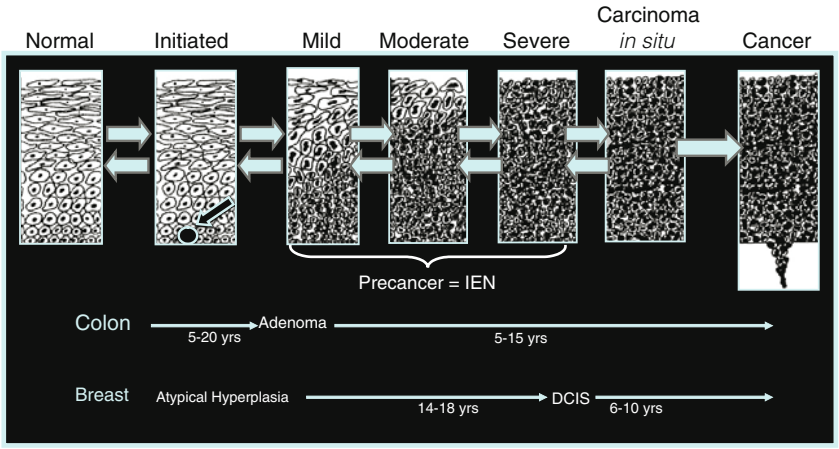


**Fig. 1** Progression of precancer to cancer in humans is a multi-year process, adapted from O'Shaughnessy et al. (2002)

## 1.1 Summary of Changes to Second Edition

Many researchers worldwide have focused their life's work to identify ways to prevent cancer. Investigators at the Arizona Cancer Center are part of this team, working to prevent cancer and to reduce the morbidity and mortality from this disease. According to the Director of the National Cancer Institute, one of the primary reasons why current knowledge and information about cancer and its prevention is not applied to the general public is due to an overload of complicated information. The dissemination of complicated information is problematic, but comprehensive information is essential to reduce the burden of cancer. The second edition of this book is designed to provide this information in the form of a comprehensive overview on the science and practice of cancer prevention for primary caregivers and the research community.

The first section of this book (Chaps. 2–10) provides information on economic issues in cancer prevention, dietary and environmental risk, immune response, drug development, the role of telemedicine technology, and cultural considerations in cancer prevention. The second section of the book (Chaps. 11–18) focuses on the prevention of specific cancers by site of origin and provides the reader with a discussion of the epidemiology, screening, and prevention of each disease, including practice guidelines as well as theories and future research directions. The book concludes with Chap. 19, a discussion of issues specific to cancer survivors. Because of the rapid advancement in cancer prevention research, several important changes have been made to the second edition of this book.

The field of cancer prevention is constantly changing as research progresses and our knowledge about cancer expands. Some chapters have only minimally been revised (Chaps. 5, 9, 12, 13 and 15); whereas other chapters have undergone moderate changes to be more current (Chaps. 2, 3, 6, 7, 16, 18 and 19), and others have undergone complete

revision and expansion (Chaps. 11, 14 and 17) to include new research findings, more in-depth information, and current clinical recommendations. An important new chapter in this revised edition is Chap. 4 (Innate and Adaptive Immune Responses to Cancer), which expands on the critical role of the immune system in cancer initiation and progression. Additionally, a second new chapter has been added (Chap. 8, Telemedicine in Cancer Prevention), which focuses on the role of telecommunications technology in the delivery of cancer prevention and control services to geographically distant locations.

## 1.2 Overview of Cancer Prevention

It is estimated that there are over 10.9 million cases of cancer diagnosed and 6.7 million deaths each year worldwide (Mackay et al. 2006). The five most common worldwide cancers, excluding non-melanoma skin cancer, include lung, stomach, breast, colorectal and liver cancer (Table 1.1). There are gender and regional differences in worldwide cancer diagnoses. Although less than 20% of the world's population lives in developed

| | Number of new cases each year |
|---|---|
| **Both sexes** | |
| Lung | 1,082,000 |
| Colon/rectum | 1,023,000 |
| Stomach | 934,000 |
| Liver | 626,000 |
| Esophagus | 462,000 |
| Bladder | 357,000 |
| Leukemia | 300,000 |
| **Males** | |
| Lung | 965,000 |
| Prostate | 679,000 |
| Stomach | 603,000 |
| Colon/rectum | 550,000 |
| Liver | 442,000 |
| Esophagus | 315,000 |
| Bladder | 274,000 |
| Leukemia | 171,000 |
| **Females** | |
| Breast | 1,151,000 |
| Cervix | 493,000 |
| Colon/rectum | 473,000 |
| Lung | 387,000 |
| Stomach | 331,000 |
| Ovary | 204,000 |
| Liver | 184,000 |
| Esophagus | 147,000 |
| Leukemia | 129,000 |
| Bladder | 83,000 |

**Table 1.1** Worldwide annual cancer incidence of the most common cancers (Mackay et al. 2006)

nations, about half (47%) of all cancer cases occur in these countries. Eighty-two percent (82%) of all liver cancer cases, 83% of all cervix cancer cases, 84% of esophagus cancer cases, and 67% of all stomach cancers occur in developing nations; whereas 76% of all prostate cancers and 65% of all colorectal cancers are diagnosed in developed nations (Mackay et al. 2006). In the United States (US), there were 1,444,920 cancers diagnosed in 2007, excluding non-melanoma skin cancer, and cancer accounted for 559,650 deaths (Jemal et al. 2007). Cancer is a major health burden in the US, accounting for one of every four deaths, and is the leading cause of death among those under the age of 65 years.

The goal of cancer prevention is to reduce the morbidity and mortality from cancer by reducing the incidence of cancer. The development of effective cancer prevention strategies has the potential to impact more than eight million cancer diagnoses and to prevent more than 5.2 million cancer-related deaths each year worldwide (Parkin et al. 1999; Pisani et al. 1999). Even with the current knowledge available, it is estimated that 60,000 cancer deaths could be prevented each year in the US alone (Curry et al. 2003). Therefore, cancer prevention is the best approach possible to reduce the burden of cancer worldwide. Cancer prevention research takes a three-pronged approach to target different aspects reducing cancer morbidity and mortality: primary, secondary and tertiary prevention.

### 1.2.1 Primary Prevention

Primary prevention involves a reduction of the impact of carcinogens, such as through administration of a chemopreventive agent or the removal of environmental carcinogens. The goal of primary prevention is to prevent a cancer from beginning to develop by reducing individual risk. Current primary prevention methods include lifestyle modification or interventions that modify risk. Primary prevention methods are best suited for those cancers in which the causes are known. There are many factors known to reduce overall cancer incidence, such as minimizing exposure to carcinogens (e.g. avoiding tobacco), dietary modification, reducing body weight, increasing physical activity, avoiding infection, or through medical intervention (surgery and/or chemoprevention). Among developed nations, the leading risk factors for cancer include an unhealthy diet or poor nutrition (responsible for 30% of cancer cases) and tobacco use (16% of cases); whereas among developing nations, poor diet/nutrition is the leading risk factor that accounts for 20% of all cancer cases, and infection is the leading risk factor that accounts for 26% of all cancer cases.

Tobacco use alone, which represents the greatest preventable cause of cancer death by far, is the cause of more than 20% of all cancer deaths worldwide each year (primarily lung cancer, but smoking also increases the risk of larynx, bladder, kidney, pancreas, stomach, colorectal, cervix, breast and many other cancers) (Mackay et al. 2006). Lung cancer, the leading cause of smoking-related cancer death (71% of all lung cancers are directly attributed to tobacco), accounts for 26 and 31% of annual cancer deaths among women and men, respectively (Jemal et al. 2007; Mackay et al. 2006). Worldwide, more than 1.4 million cancer deaths each year are attributed to tobacco exposure (Mackay et al. 2006).

Tobacco also causes an additional 1.7 million deaths from cardiovascular disease, and nearly 1 million deaths from coronary obstructive pulmonary disease (COPD) each year (Mackay et al. 2006). This results in more than four million deaths worldwide each year that can be directly attributed to tobacco use or exposure. Smoking cessation is now known to reverse the risk of cancer. Benefits from quitting smoking begin within the first year of cessation and continue to increase. The risk of lung, oral and laryngeal cancers can be reduced by up to 60% within 10–15 years after smoking cessation, and the reversal appears to continue to improve as the time since cessation increases (Dresler et al. 2006). Primary tobacco prevention efforts include cessation support programs (behavioral and pharmacologic), smoke-free public policies, and very importantly, efforts to reduce the initiation of the use of any form of burnt and smokeless tobacco, all of which are carcinogenic and deadly.

Many cancers are now known to be directly attributable to viral infections (e.g. human papillomavirus infection is a necessary factor in the development of cervical cancer; *Helicobacter pylori* is an initiator and promotor for gastric cancer). In this revised edition, a new chapter exploring the role of the immune system in cancer has been included (Chap. 4). Additionally, the role of diet, nutrition and maintaining a healthy body weight is critical to reducing cancer risk. These factors are described in more detail in Chap. 3.

Modification of tobacco use and other risk factors (i.e. dietary or environmental) could have a very significant impact on worldwide health through primary prevention efforts. Unfortunately, primary prevention research and efforts are largely underfunded. In the US and Europe, less than 10% of all cancer research funding is dedicated to cancer prevention efforts (Mackay et al. 2006). This lack of prioritization results in delays in improving and delivering early detection and prevention strategies that have the potential to save millions of lives.

### 1.2.2 Secondary Prevention

Secondary prevention involves the concept of a precancerous lesion, or abnormal changes that precede the development of malignancy. Secondary prevention involves screening and early detection methods (e.g. mammogram, prostate-specific antigen test, colonoscopy) that can identify abnormal changes before they become cancerous, thereby preventing the cancer before it fully develops. In general, secondary prevention is associated with the removal of an IEN or precancerous lesion (e.g. ductal carcinoma in situ, adenoma, hyperplasia). In some cases, secondary prevention can involve the treatment of precancerous lesions in an attempt to reverse carcinogenesis (e.g. cause the lesion to regress). Secondary prevention is described in more detail specific to each disease site (Chaps. 11–18) in this book.

### 1.2.3 Tertiary Prevention

Tertiary prevention involves the care of established disease and the prevention of disease-related complications and often encompasses the treatment of patients at high risk of developing a second primary cancer. Tertiary prevention, often referred to as

cancer control, involves a variety of aspects of patient care, such as quality of life, adjuvant therapies, surgical intervention and palliative care. These efforts are described in more detail in Chap. 19.

## 1.3 Multi-Step Carcinogenesis Pathway

Prevention of cancer requires an understanding of the process of cancer initiation and the steps to progression of disease. This process is referred to as carcinogenesis, a process of genetic alterations that causes a normal cell to become malignant. Cancer prevention involves the identification and classification, as well as interventions, for the regression or removal of precursor lesions, often referred to as intraepithelial neoplasia (IEN), before they can become cancerous. As shown earlier in Fig. 1, the process of carcinogenesis may take many years. In the case of colorectal cancer, it may take up to 35 years from the first initiated colonic mucosal cell to an adenomatous polyp to develop invasive cancer. The same is true for prostate cancer, which progresses over as many as 40–50 years from mild to moderate, then severe intraepithelial neoplasia, to latent cancer (see Chap. 15).

The vast majority of current treatment modalities are used to treat far advanced and/or metastatic cancers; however, now that it is possible to identify IENs for virtually every solid tumor type, lifestyle changes, simple surgical procedures and chemopreventive agents can be used to impede the development of these potentially dangerous precancerous lesions (Fig. 2). For example, multiple lifestyle changes, taken together, could profoundly reduce the risk of the first initiated cell progressing to mild dysplasia. This would include reducing dietary fat intake, increasing the number of servings of fruits and vegetables, minimizing alcohol intake, tobacco exposure cessation and markedly increasing physical activity. Furthermore, the addition of an effective chemoprevention agent, such as multivalent human papillomavirus vaccines to reduce the risk of cervical carcinoma, or tamoxifen for women at very high risk of breast cancer, can significantly reduce cancer risk (Fisher et al. 1998; Schiffman et al. 2007). Thus, the concept of cancer prevention is now evolving into the mainstream of cancer therapeutics.

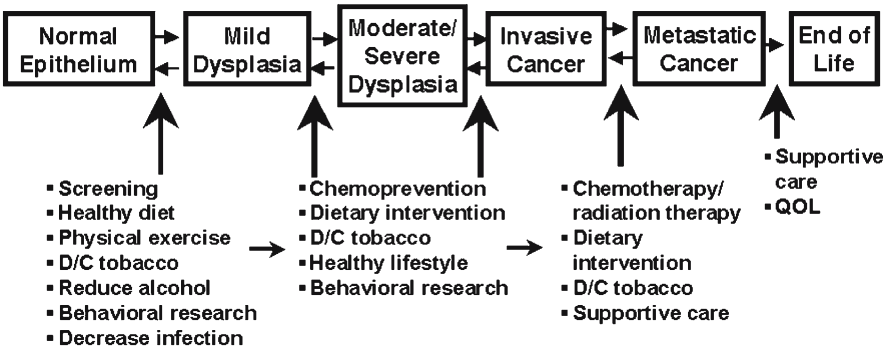


**Fig. 2** Multi-step carcinogenesis pathway (adapted from Alberts et al. 1999)

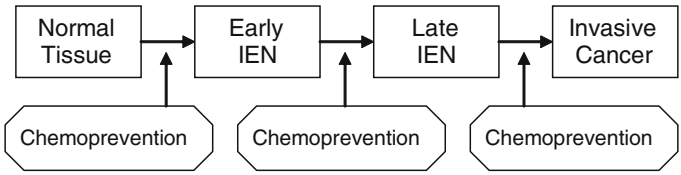


**Fig. 3** Chemoprevention of intraepithelial neoplasia (IEN)

The process of carcinogenesis involves multiple molecular events over many years to evolve to the earliest dysplastic lesion or IEN. This multi-year process provides numerous opportunities to intervene with screening, early detection, surgical procedures, and chemoprevention (i.e. the use of specific nutrients and/or chemicals to treat IENs and/or delay their development) (Sporn 1976). Figure 3 presents a simplified model of the concept that an effective chemopreventive agent could prevent IEN growth, progression or, ultimately, invasion through the tissue basement membrane

## 1.4 Cancer Prevention Research

The importance of conducting and participating in clinical trials cannot be understated. Every person is at risk of genetic mutations that may lead to cancer. Due to endogenous or exogenous factors, every human body has undergone genetic alterations. For many individuals, these initiating factors are the early steps to the development of IEN or cancer. The time period from the first initiated cell to the time of cancer is estimated to be approximately 20 years. As described earlier, the early steps towards cancer occur over time, which means that literally millions of individuals are currently in some phase of undetected cancer progression that will ultimately result in their death (Wattenberg 1993).

Cancer prevention trials are research studies designed to evaluate the safety and effectiveness of new methods of cancer prevention or screening. The focus of cancer prevention research can involve chemoprevention (including vaccination), screening, genetics, and/or lifestyle changes (e.g. diet, exercise, tobacco cessation). Cancer chemoprevention research differs from treatment research in several important ways as shown in Table 1.2. Cancer chemoprevention trials generally are performed in relatively healthy volunteers who have well-documented IENs (e.g. colorectal adenomas, bladder papillomas, ductal carcinoma in situ, actinic keratosis in the skin) or at increased risk due to genetic or other factors. These trials are usually double-blind (i.e. both physician and participant do not know the assigned treatment), placebo-controlled and involve a few thousand to tens of thousands of randomized participants. As opposed to cancer treatment phase III trials that rarely extend beyond 5 years in duration, cancer chemoprevention trials often take many years to complete and are extremely costly. The high cost of cancer prevention trials and the need to develop reliable and meaningful intermediate endpoints are significant barriers that must be overcome. Cancer prevention clinical trials take between 5 and 10 years (or more) to complete,

**Table 1.2** Cancer chemoprevention versus cancer treatment phase III trials (adapted from Alberts et al. 2004)

| Characteristic | Cancer chemoprevention trials | Cancer treatment trials |
|---|---|---|
| Participants | Relatively healthy volunteers with IENs or at moderate/high risk | Patients diagnosed with invasive cancer |
| Trial design | Commonly double-blind, placebo controlled | Unblinded to both patient and investigator |
| Dosage | Minimize dose, emphasize safety | Maximize dose, emphasize efficacy |
| Toxicity | Dosage changes with any toxicity, concern for long-term use of agent | Moderate toxicity acceptable, less concern with toxicity due to severity of disease |
| Adherence | Concern for "drop ins" due to media or hype | Concern for "drop outs" due to toxicity |
| End point | Surrogate biomarkers; cancer incidence | Mortality |
| Sample size | A few thousand to several thousand participants | A few hundred to a thousand participants |
| Trial duration | Often 5–10 years | Several months to several years |

and require thousands of participants. In US dollars, the cost to complete large-scale trials (10,000 participants or more) is in the $100–$200 million range and, of course, may not always result in the discovery of an effective prevention strategy.

Research to develop and implement effective cancer prevention and control interventions lags in funding relative to its potential impact on reducing the cancer burden. Despite the known cancer-causing effects of tobacco use, few non-nicotine medications are currently approved by the US Food and Drug Administration (FDA) for smoking cessation, though others are in the pipeline, and these existing medications achieve smoking cessation quit rates that are 25% at best. Since many health care organizations do not include smoking cessation medications as a covered benefit, the incentive for pharmaceutical companies to prioritize the development of smoking cessation medications is not high – thus fostering a negative feedback loop that disincentivizes health care organizations from covering medications because the effectiveness of those medications is low. Similarly, pharmaceutical companies have traditionally been unwilling to invest in the development of chemopreventive agents because of the required length of time, size and cost of phase III confirmatory trials. Furthermore, companies are concerned about uncovering unexpected, life threatening toxicities that may be observed with the long-term exposure required for many cancer prevention intervention strategies. This can have an extremely negative impact on safety profiles of approved drugs (e.g. twice daily dosing of COX-2 inhibitors increased cardiovascular events by 4–5% in at least two blinded prospective trials).

The phases of investigation in cancer prevention research trials (phase I through IV trials) are described in more detail in Chap. 9. Briefly, phase I trials take place after an agent has demonstrated activity with low toxicity in preclinical models. Phase I trials are

brief (several weeks), preliminary research studies in healthy humans to determine safety of an agent. Phase II trials may be either non-randomized (phase IIa) or randomized, double blinded, placebo controlled (phase IIb) and are of longer duration (several weeks to months). The goals of phase II trials are to determine the activity of an agent in IEN or a surrogate endpoint and to further evaluate safety. Phase III trials generally are large, double-blind, controlled randomized trials to evaluate the effectiveness and safety of an agent in a sample of the target population. Often, cancer incidence is the primary endpoint in phase III prevention studies. For a chemopreventive agent to be used in a phase III research setting, it must meet several criteria. The agent must have strong data supporting its mechanistic activity and there must be preclinical efficacy data from appropriate animal models. If the chemopreventive agent is a nutrient, there must be strong epidemiologic data supporting its potential effectiveness and it must have demonstrated safety and activity in phase II trials. Phase III trials of novel chemopreventive agents should not be performed in the absence of a fundamental understanding of its mechanism of action. Finally, phase IV trials are focused on the dissemination of the phase III trial results into the population and the efficacy of these interventions in a real-world setting. Inadequate funding and insufficient attention has been given to vitally important dissemination studies, leading to underutilization of effective chemoprevention strategies.

When the mechanism of activity of a putative chemoprevention agent has not been explored in the setting of broad populations, the results of phase III trials can be alarming. Two examples of this include the results of the Finnish Alpha-Tocopherol, Beta-Carotene (ATCB) Trial and the University of Washington Carotene and Efficacy Trial (CARET). Both of these phase III trials used relatively high doses of beta-carotene as compared to placebo in heavy smokers to reduce the incidence of and mortality from lung cancer (1994; Omenn et al. 1996). Unfortunately, both trials found that the beta-carotene intervention was asociated with a 18–28% increase in lung cancer incidence and an associated increase in mortality. Perhaps the reason for these unexpected and extremely unfortunate results relates to the fact that at high beta-carotene concentrations in the setting of high partial pressures of oxygen (e.g. as achieved in the lung) and in the presence of heat (e.g. as achieved in the lung with cigarette smoking), beta-carotene can become an autocatalytic pro-oxidant (versus its usual role as an anti-oxidant) (Burton and Ingold 1984).

The design of chemoprevention phase III trials must be founded on a hypothesis that is soundly based on the mechanism of action of the agent, epidemiologic data and its preclinical efficacy. The population to be enrolled to a phase III prevention trial must be of relatively high cancer risk, to assure that there will be a sufficient number of events (e.g. precancers or cancers) to compare the treatment to the control. Phase III prevention trials should include both intermediate (e.g. IEN) and long-term (e.g. cancer) endpoint evaluations. Most importantly, the endpoint analyses should be planned in advance, including well-defined and well-powered primary and secondary analyses.

One example of a potentially high-impact phase III chemoprevention trial is the Breast Cancer Prevention Trial with Tamoxifen (BCPT) (Fisher et al. 1998). Healthy women at increased risk of breast cancer were randomized to either tamoxifen (20 mg per day) or placebo for up to 5 years. Tamoxifen was selected for this trial because of its well-documented mechanism of action (i.e. binding to the estrogen receptor to prevent estrogen's effect on tumor cell proliferation), its strong safety profile in the

setting of adjuvant breast cancer therapy, and its activity in the prevention of contralateral breast cancer in patients with stage I/II breast cancer. After 69 months of follow up, tamoxifen was found to be associated with an overall 49% reduction in the risk of invasive breast cancer (Fisher et al. 1998). The benefit of breast cancer risk must be balanced with its toxicities, which include a greater than twofold increase in early stage endometrial cancer and an increased incidence of deep vein thrombosis and pulmonary embolism. Since the publication of these results, much discussion has led to the identification of women who would most benefit from treatment with tamoxifen. Certainly, women who are at significantly increased breast cancer risk, have already undergone a hysterectomy, and who at lower risk for thrombophlebitis (e.g. due to higher levels of physical activity, lack of obesity) would be good candidates for this intervention. Furthermore, there has been a relative lack of dissemination of this information to both primary care physicians and the population, resulting in limited or inappropriate tamoxifen usage (Freedman et al. 2003). More recently, the results of the phase III Study of Tamoxifen and Raloxifene (STAR) revealed equivalent activity of tamoxifen as compared to raloxifene for the reduction of breast cancer risk among postmenopausal women at moderately increased risk (Vogel et al. 2006). Raloxifene was associated with an improved safety profile (e.g. lower thromboembolic events and cataracts), leading to its approval as a chemopreventive agent with the FDA. Only time will tell if these results will lead to increased chemoprevention utilization.

The translation of research findings to the clinic is the ultimate goal of cancer prevention research. Chemoprevention agents or screening modalities must be acceptable to the target population that would benefit from such interventions. For example, the ideal chemoprevention agent would have a known mechanism of action and would have no or minimal toxicity, high efficacy, be available orally or topically, have an acceptable treatment regimen, and would be inexpensive. Similarly, screening or early detection modalities should be minimally invasive, have high sensitivity and specificity, and be acceptable to the target population. Interventions that fail to maintain adequate adherence or that have high attrition rates during phase III trials will likely also not be acceptable to the patient in clinical practice.

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# Assessing Human and Economic Benefits of Cancer Prevention

2

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It is critically important to discuss and attempt to quantify the human and economic value of cancer prevention. The purpose of this chapter is to provide an overview of the ways in which this value can be defined and assessed. As will be described in much more detail in subsequent chapters, cancer prevention takes many forms. At the individual level, virtually all prevention activities involve: (1) engaging in particular behaviors (e.g., following screening and immunization recommendations, taking tamoxifen for secondary prevention of breast cancer); (2) avoiding particular behaviors (e.g., sunbathing, smoking); or (3) changing particular behaviors once they have become habitual or routine (e.g., quitting smoking, lowering dietary fat). Each of these prevention behaviors, or the lack of them, can have short- and long-term health, quality of life, and/or economic consequences.

## 2.1 Outcomes Assessment

In order to discuss the impact of cancer and, hence, the substantial benefits of preventing it, it is necessary to define *outcomes*. A conceptual framework articulated by Kozma and colleagues places outcomes into three categories: economic, clinical, and humanistic (Kozma et al. 1993). Economic outcomes are changes in the consumption and production of resources caused by disease or intervention, such as cancer prevention. The changes may be direct (e.g., cost of a medication) or indirect (e.g., early retirement due to reduced productivity). Clinical outcomes are the medical events that occur as a result of the condition or its treatment as measured in the clinical setting. Humanistic, or patient-reported, outcomes include condition or intervention-related symptoms and side effects, treatment satisfaction, health status, and self-assessed function and well-being, or health-related quality of life.

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The major cancer clinical trial cooperative groups in North America and Europe have recognized the importance of this outcomes triad in evaluating and improving the net benefit of cancer therapy (Bruner et al. 2004). Humanistic and economic outcomes, which are the focus of this chapter, are increasingly being incorporated into clinical trials (Lipscomb et al. 2004). In addition, the importance of outcomes assessment in cancer was reinforced with the National Cancer Institute's (NCI) establishment of its Outcomes Research Branch in 1999 (Lipscomb and Snyder 2002) and the Cancer Outcomes Measurement Working Group in 2001 (Lipscomb et al. 2005). According to the NCI, "outcomes research describes, interprets, and predicts the impact of various influences, especially (but not exclusively) interventions on 'final' endpoints that matter to decision makers: patients, providers, private payers, government agencies, accrediting organizations, or society at large" (Lipscomb and Snyder 2002).

## 2.2 Humanistic Outcomes

As mentioned above, humanistic or patient-reported outcomes (PROs) include a wide range of health-related concepts or constructs. According to the US Food and Drug Administration (FDA), PROs are on a continuum from the purely symptomatic (e.g., pain intensity) to more complex aspects of functioning (e.g., ability to perform activities of daily living) to much more complex concepts (e.g., quality of life) (FDA 2006). Since many cancer prevention activities are aimed at populations rather than individual patients, the term PRO in the context of this chapter may seem too narrow; however, the intent is to convey the importance of capturing individual's health and health care perceptions and experiences through self-report. The PRO that has increasingly garnered the most attention, particularly in regard to drug therapy (Willke et al. 2004; European Medicines Agency 2005), is health-related quality of life, which will be a primary focus of this section.

Quality of life is a commonly used term that usually conveys a general feeling rather than a specific state of mind. A person's quality of life, or subjective well-being, is based on personal experience and expectations, that affect and can be influenced by many factors, including standard of living, family life, friendships, and job satisfaction (Sirgy et al. 2006). Although health can impact these factors, health care is not directly aimed at enhancing them. Studies of health outcomes use the term *health-related quality of life* to distinguish health effects from the effects of other important personal and environmental factors. There is growing awareness that in certain diseases, such as cancer, or at particular stages of disease, health-related quality of life may be the most important health outcome to consider in assessing the effect of interventions (Staquet et al. 1992).

In much of the empirical literature, explicit definitions of health-related quality of life are rare; readers must deduce its implicit definition from the manner in which its measurement is operationalized. However, some authors have provided definitions. For example, Revicki and colleagues define health-related quality of life as "the subjective assessment of the impact of a disease and treatment across physical, psychological, social, and somatic domains of functioning and well-being" (Revicki et al. 2000, p. 888).

Ferrans (2005) has provided a useful overview of various definitions and conceptual models of health-related quality of life. Definitions may differ in certain respects, but an important conceptual characteristic they share is multidimensionality. Essential dimensions of health-related quality of life include:

- Physical health and functioning
- Psychological health and functioning
- Social and role functioning

In addition, disease- and/or treatment-related symptomatology (e.g., pain), general well-being, and spiritual well-being are sometimes assessed. The latter is more likely to be included in measures developed for conditions that have the potential to impact not only quality of life but length of life as well (e.g., cancer). For example, the four-dimensional model that provides the framework for the cancer-related quality of life questionnaires developed at the City of Hope National Medical Center includes spiritual well-being along with physical, psychological, and social well-being (Grant et al. 2004).

## 2.3 Measuring Health-Related Quality of Life and Other Patient-Reported Outcomes

Although PROs such as health-related quality of life are subjective, they can be quantified (i.e., measured) in a uniform and meaningful way. The quality of the data-collection tool is the major determinant of the quality of the results. Psychometrics refers to the measurement of psychological constructs, such as knowledge, attitudes, and well-being. It is a field of study concerned with the proper development and testing of instruments (e.g. questionnaires) so that confidence can be placed in the measurements obtained. Two of the most commonly assessed psychometric properties are reliability and validity. Briefly, reliability refers to the consistency, stability, or reproducibility of scores obtained on a measure; validity reflects whether the instrument actually measures what it is purported to be measuring. More thorough discussions of these properties are provided elsewhere (Streiner and Norman 2003; Frost et al. 2007). Anyone planning to use PRO measures in cancer prevention research or clinical practice should confirm that there is adequate evidence to support the reliability and validity of the measures chosen.

Cullen and colleagues, in their review the short-term quality of life impact of cancer prevention and screening activities, addressed ways in which outcomes have been assessed through the use of new and existing measures (Cullen et al. 2004). Since many of the outcomes were exclusively psychological states (e.g., anxiety, relief) or symptoms, they cannot be considered assessments of health-related quality of life, Measures of health-related quality of life should include, at a minimum, the three essential dimensions (i.e., physical, psychological, social) recognized as comprising it. Nevertheless, the review by Cullen and colleagues and another by Mandelblatt and Selby (2005) provide important insight into the research that has been conducted to assess the short-term patient-reported

consequences of clinical preventive services such as chemoprevention, genetic testing and counseling, and screening. Knowledge of these consequences is critical in attempting to understand and act upon the factors that may affect participation in prevention-related activities. Although it remains an empirical question, it appears that the predominantly transient negative consequences of participating in cancer prevention activities would be readily offset by the positive long-term outcomes (e.g., avoidance of quality of life losses resulting from cancer-related morbidity). As asserted by Badia and Herdman (2001), preventive interventions are unlikely to lead to gains in quality of life, but should prevent or delay reductions in quality of life over time.

A specific example of PRO assessment in the context of cancer prevention is provided by the National Surgical Adjuvant Breast and Bowel Project (NSABP) Study of Tamoxifen and Raloxifene (STAR) P-2 Trial (Land et al. 2006). The STAR P-2 Trial was designed to evaluate the relative efficacy of the two study drugs in reducing the incidence of invasive breast cancer in high-risk post-menopausal women. The investigators used a number of measures to compare patient outcomes by treatment arm, including the Center for Epidemiological Studies Depression Scale (CES-D), the Medical Outcomes Study (MOS) Sexual Activity Questionnaire, a 36-item symptom checklist, and the MOS 36-Item Short-Form Health Survey (SF-36). This battery of multiple instruments and scales enabled the investigators to assess the PROs they felt were most relevant in the target population. The health-related quality of life end points were the physical (PCS) and mental component summary (MCS) scores of the SF-36. The SF-36 will be discussed in more detail below.

There are hundreds of PRO instruments currently available (Bowling 1997; McDowell 2006), some of which have been developed for use in people with cancer (Bowling 2001; Donaldson 2004) or for individuals undergoing cancer screening (Mandelblatt and Selby 2005). The Psychosocial Effects of Abnormal Pap Smears Questionnaire (PEAPS-Q) (Bennetts et al. 1995) and the Psychosocial Consequences Questionnaire for abnormal screening mammography (PCQ-DK33) (Broderson et al. 2007) are examples of PRO measures specifically developed for cancer-related clinical preventive services. However, the vast majority of available PRO measures were developed for use in people already experiencing disease and/or disability. The value of these measures in the context of cancer prevention is that they provide quantitative evidence of the losses in functioning and well being that may be avoided by effective prevention strategies. A primary distinction among PRO instruments, particularly measures of health-related quality of life, is whether they are specific or generic.

### 2.3.1 Specific Measures

The pioneering work of Karnofsky and Burchenal in the 1940s that produced the Karnofsky Performance Scale recognized the need to assess the patient's functional status in the context of cancer chemotherapy (Karnofsky 1949). This tool, which was designed for clinician assessment of observable physical functioning, is still used today. It was one

| EORTC QLQ-C30 | FACT-G |
|---|---|
| Physical functioning | Physical well-being |
| Role functioning | Social/family well-being |
| Cognitive functioning | Emotional well-being |
| Emotional functioning | Functional well-being |
| Social functioning | |
| Fatigue | |
| Global quality of life | |
| Nausea and vomiting | |
| Pain | |

**Table 2.1** Domains/dimensions addressed by the FACT-G and EORTC QLQ-C30

of the first steps in the development of patient-centered and, ultimately, patient-reported outcome measures. Since then, a considerable amount of time and effort has been invested in the development of cancer-specific instruments for use in clinical trials and routine patient monitoring. Another of these instruments is the Q-TWiST (Quality-Adjusted Time Without Symptoms and Toxicity), which addressed both quality and quantity of time following cancer treatment (Gelber et al. 1993). Other examples are the EORTC QLQ-C30 (Aaronson et al. 1993) and the Functional Assessment of Cancer Therapy-General (FACT-G) (Cella et al. 1993). The European Organization for Research and Treatment of Cancer (EORTC) has worked extensively in the area of instrument development (www.eortc.be/home/qol). In addition, the developers of the FACT-G have a broad array of cancer-specific instruments available (www.facit.org). Table 2.1 lists the dimensions covered by the EORTC QLQ-C30 and the FACT-G. Each of these instruments was designed to be supplemented with additional modules or scales aimed at specific cancer patient subgroups.

Cancer-specific instruments such as these are intended to provide greater detail concerning particular outcomes, in terms of functioning and well-being, uniquely associated with a condition and/or interventions to treat or prevent it. Disease- or condition-specific instruments may be more sensitive than a generic measure to particular changes in health-related quality of life secondary to the disease or its treatment. For example, the Functional Assessment of Cancer Therapy (FACT) subscales, such as the neurotoxicity subscale (FACT-NTX,) address specific concerns (e.g., finger numbness, difficulty buttoning), which would not be addressed in a generic instrument. In addition, specific measures may appear to be more clinically relevant to patients and health care providers since the instruments address issues directly related to the disease (Guyatt et al. 1993). However, a concern regarding the use of only specific instruments is that by focusing on the specific impact of a disease or its treatment, the general or overall impact on functioning and well-being may be overlooked. Therefore, the use of both a generic and a specific instrument may be the best approach. This was the approach taken by the developers of the UCLA Prostate Cancer Index, which covers both general and disease-specific (e.g., sexual, urinary and bowel function) concerns (Litwin et al. 1998).

### 2.3.2 Generic or General Measures

Since primary cancer prevention involves avoiding the occurrence of disease, general measures may be more applicable in that context. Generic, or general, instruments are designed to be applicable across a wide variety of populations, across all diseases or conditions, and across different medical interventions (Patrick and Deyo 1989). The two main types of generic instruments are health profiles and preference-based measures.

*Health Profiles*. Health profiles provide multiple outcome scores representing individual dimensions of health status or health-related quality of life. An advantage of a health profile is that it enables clinicians and/or researchers to measure the differential effects of a disease state or its treatment on particular dimensions. The most commonly used generic instrument in the world today is the SF-36 (www.sf36.org), which was used as a component of the STAR P-2 Trial discussed above. The SF-36 includes eight multi-item scales (Table 2.2) which address a wide array of dimensions (Ware and Sherbourne 1992). Each of the scale scores can range from 0 to 100, with higher scores representing better functioning or well-being. It is brief (it takes about 10 min to complete) and its reliability and validity have been documented in many clinical situations and disease states (Ware 2000). A means of aggregating the items into physical (PCS) and mental component summary (MCS) scores is available (Ware 1994). However, the SF-36 does not provide an overall summary or index score, which distinguishes it from the preference-based measures.

*Preference-Based Measures*. For health-related quality of life scores to be most useful as an outcome in economic analysis, they need to be on a scale anchored by 0.0 (i.e., death) and 1.0 (i.e., perfect health). The values for the health states represented on the scale reflect the relative desirability or preference level for individual health states as judged by population- or patient-based samples. Although one can undertake direct preference measurement, a number of preference-based instruments are already available for which the health state preferences have been derived empirically through population studies. Examples include the Health Utilities Index (HUI) (www.healthutilities.com), the EuroQol Group's EQ-5D (www.euroqol.org), and the SF-6D (www.sf36.org). The SF-6D was developed to provide a preference-based overall summary or index score for data collected with the SF-36 (Brazier et al. 2002). The domains addressed by each of these instruments are listed in Table 2.2.

*Quality-Adjusted Life Years (QALYs)*. The preference-based instruments described above are administered to assess respondents' self-reported health status, which is then mapped onto the instrument's multiattribute health status classification system producing a health-related quality of life score on the 0.0–1.0 scale. Scores on this scale, which may represent the health-related consequences of disease or its treatment, can be used to adjust length of life for its quality resulting in an estimate of *quality-adjusted life years* (QALYs). QALYs integrate in a single outcome measure the net health gains or losses, in terms of both quantity and quality of life. The metric of life-years saved (LYS) is not sufficient since death is not the only outcome of concern; health-related quality of life changes can occur with or without changes in life years. The QALY approach assumes that one year in full health is scored 1.0 and death is 0.0. Years of life in less than full health are scored as less

**Table 2.2** Domains included in selected generic instruments

| **SF-36** | |
|---|---|
| Physical functioning | |
| Role limitations due to physical problems | |
| Bodily pain | |
| General health perceptions | |
| Vitality | |
| Social functioning | |
| Role limitations due to emotional problems | |
| Mental health | |
| **Quality of well-being scale (QWB)** | |
| Mobility | |
| Physical activity | |
| Social activity | |
| Symptoms/problems | |
| **Health utilities index (HUI)** | |
| **HUI2** | **HUI3** |
| Sensation | Vision |
| Mobility | Hearing |
| Emotion | Speech |
| Cognition | Ambulation |
| Self-care | Dexterity |
| Pain | Emotion |
| Fertility | Cognition |
| | Pain |
| **EQ-5D** | |
| Mobility | |
| Self-care | |
| Usual activity | |
| Pain/discomfort | |
| Anxiety/depression | |
| **SF-6D** | |
| Physical functioning | |
| Role limitation | |
| Social functioning | |
| Mental health | |
| Bodily pain | |
| Vitality | |

than 1.0 QALY. For example, based on a review by Tengs and Wallace, a year of life with small-cell lung cancer after the disease has progressed is equal to 0.15 QALY (Tengs and Wallace 2000).

QALYs can be a key outcome measure, especially in diseases such as cancer, where the treatment itself can have a major impact on patient functioning and well-being. Although the QALY is the most commonly used health outcome summary measure, it is not the only

one (Gold et al. 2002). Other conceptually equivalent outcomes include *years of healthy life* (YHL), *well years* (WYs), *health-adjusted person years* (HAPYs), and *health-adjusted life expectancy* (HALE). As observed by Ubel, without an outcome measure such as QALYs, it would be impossible to compare the relative cost-effectiveness of life-prolonging versus life-enhancing interventions, much less interventions that do both (Ubel 2001). The remainder of this chapter discusses the economic issues and methodologies relevant to cancer prevention.

## 2.4 Economic Outcomes and Cancer

Prevention of cancer renders an economic benefit for society by reducing the amount of resources necessary for the treatment of cancer. The NCI reports that cancer treatment accounted for $41 billion in 1995, just under 5% of total U.S. spending for medical treatments (NCI 2004). By investing in cost-saving cancer prevention modalities, more resources may be available for the overall health care system. Johnson and colleagues estimate that 53% and 13% of the medical expenditures for persons with lung cancer and chronic obstructive pulmonary disease are attributable to smoking, respectively (Johnson et al. 2003). Substantial medical resources would become available, if smoking were reduced.

Economic benefits rendered by improving health go beyond the costs associated with medical treatments. The economic benefits of cancer prevention include decreases in the frequency of health-related disruptions in productive activity, such as lost work days. By promoting health, cancer prevention reduces the need for assistance with personal care services and allows greater intangible benefits, like dignity, autonomy, and individuality. Simply put, prevention is better than cure because, as stated by Thomas Adams, a seventeenth century physician, it saves the labor of being sick.

Although the economic benefits of cancer prevention are widely acknowledged, especially by NCI, there is a paucity of evidence regarding these benefits. The information regarding economic outcomes that is available is rarely translated for and applied to evidence-based medical decision making. As a result, cancer prevention is often inefficiently utilized. Researchers who study the economic outcomes of cancer prevention provide valuable information to individuals and institutions, who may fail to consider the full scope of the economic benefits (Fryback and Craig 2004). For example, the vaccination of girls against human papillomavirus (HPV) may be justified on grounds of improved health and cost savings; however, the case for boys, who may be future carriers of the cancer-causing virus, require deliberation over whether the marginal benefits is worth the high cost in terms of cancer resources (Elbasha et al. 2007).

Decision makers at the individual, institutional, or governmental levels require evidence on economic outcomes of cancer prevention to improve their ability to make informed choices with regard to prevention activities, thereby maximizing limited resources. In this section, we describe core concepts in economic outcomes research and provide examples to illustrate their importance.

## 2.5 Defining and Measuring Economic Outcomes

Every cancer prevention strategy entails a change in the use of scarce resources, also known as an economic outcome. If we were to list the resources necessary to produce an intervention and the resources saved due to the intervention, we would have a description of the net bundle of resources attributable to the intervention. This bundle is known as the intervention's *opportunity cost*. Once the intervention is undertaken, the opportunity to use these resources differently is lost. Consideration of the economic outcomes associated with interventions is important for individuals and institutions that practice evidence-based medicine.

Economic outcomes, changes in resources due to an intervention, may be categorized by system, path, and flow. Resources from the medical system, such as physician time, medications, and hospital beds, are distinguished from non-medical resources, such as community, familial, and personal goods. For example, fuel consumption by an ambulance is a medical outcome, whereas fuel for personal transportation to a clinic is a non-medical outcome. Medical and non-medical resources are differentiated, because each system faces different budgetary constraints.

Economic outcomes are also separated by their path, whether they are directly related to an intervention or indirectly related through a change in health caused by the intervention. For example, a nicotine patch may be consumed as part of a smoking cessation intervention, therefore a direct economic outcome of the intervention. The patch may change smoking-related behaviors, such as smoking breaks at work. Changes in productivity are indirect economic outcomes of the intervention. The direct and indirect outcomes are components of the smoking cessation program's opportunity cost. The concept of indirect and direct economic outcomes is unrelated to the accounting term "indirect costs" referring to overhead or fixed costs.

Economic outcomes represent an inflow of resources through consumption or an outflow of resources through production. Patients directly consume medical resources over the course of an intervention, but patients are also producers of resources. Smoking cessation programs change the consumption of resources, such as cigarettes, nicotine replacement medications, and counseling. These programs may also affect the productivity of individuals, either by making their lives more productive, or by extending their productive lives. When considering the economic benefits of cancer prevention, the effect on the consumption of current resources may be small compared to the benefits in terms of productive activities. Economic outcomes can be characterized as medical or non-medical, direct or indirect, and an inflow or outflow of resources.

*Unit of Economic Outcomes*. Economic outcomes are best measured in natural units. Natural units often appear in the form of number of hours, quantities of a specific medication, or distance traveled. Natural units describe the changes in the inflow and outflow of resources related to the intervention. Clinical-economic trials, which are randomized controlled trials that prospectively collect economic endpoint data, provide the strongest evidence on economic outcomes, because these trials randomly assign alternatives to participants to identify causality. In a prospective substudy of a randomized clinical

trial, Sculpher and colleagues (Sculpher et al. 2000) evaluated alternative drug therapies, reltitrexed and fluorouracil with folinic acid, for advanced colorectal cancer based on the number of trips made to and from the hospital and the time lost from usual activity over the therapy period. In their study, they examined medical records for medical resource consumption data and self-report data to assess travel mode, distance, and time. This is an excellent example of a clinical-economic trial that collected economic endpoint data in natural units. These natural units can be translated into monetary values according to the perspective of the decision maker.

In economics, price is cost plus marginal profit, but outside of economics, price is often confused with cost and charges. Price represents the market value of a good, if sold. If the objective of the study is to predict revenue (or expenditure), natural units are to be translated using market values (i.e., prices). Market values fluctuate over time or region, according to market forces. If the objective is to predict cost of an intervention, natural units are to be translated according to the cost of producing those resources. The cost of producing resources may also depend upon market price of the inputs necessary for resource production. For example, a mammogram may cost a provider organization $50 to produce, but they set a price of $75, because that is what the market will bear. The difference between price and cost, $25, is the marginal profit for the health organization. The inclusion and extent of marginal profit in the translation of natural units into monetary values depends on the perspective of the decision maker. The reasonable amount of profit on a mammogram for a clinic is up for interpretation.

A charge is a payment of a claim, the rightful reimbursement for the provision of goods and services according to a contract. It is neither a price nor a cost, because of its dependence on the contractual relationship between institutions. For example, managed care organizations often shift funds between services, overcharging for specialist visits to subsidize mammography under the same contract. Unlike prices, the charge for one resource may depend on the charges for other resources under the same contract. This dependent relationship makes it difficult to interpret endpoints measured through charges. However, it is well-documented that charges exceed cost in most circumstances. The economic outcomes may be represented in monetary terms, such as costs, prices, and charges, depending on perspective. However, it is important the perspective of the translation (i.e., unit of analysis) match the perspective of the decision maker, so that they may practice evidence-based medicine.

*Perspective of Economic Outcomes*. The monetary value of an economic outcome depends on the perspective of the decision maker (e.g., individual, institutional, societal). Individuals face different prices (or costs) than institutions, so they translate natural units into monetary values differently. The societal perspective considers the economic outcome borne by all individuals, and uses market value to translate natural units into monetary values. For example, the monetary value of an hour of a physician's time may be equal to a copayment from a patient's perspective, an institution-specific wage from a managed care organization's perspective, or a market wage from the societal perspective.

Differing perspectives may lead decision makers to disagree on policies regarding cancer prevention. Smoking cessation programs have medical and non-medical economic outcomes. Medical outcomes attributable to certain programs may entail a monetary loss from the perspective of a managed care organization. After incorporating the non-medical

outcomes, the programs may appear to save resources from the societal perspective. Disagreement between governmental and institutional decision makers over the economic consequences of smoking cessation programs are related to the translation of natural units into monetary values. Furthermore, societal and institutional perspectives often disagree about the inclusion of institutional profit in the translation.

## 2.6 Evaluative and Descriptive Analyses in Cancer Prevention

The economic benefits of cancer prevention are commonly described as a matter of investment in health (Wagner 1997). By investing medical resources in cancer prevention today, substantial economic benefits may accrue in the future. The purpose of evaluative analyses in cancer prevention is to examine the economic and health outcomes of alternative interventions, so that decision makers may better understand the potential impact of cancer prevention. There are four forms for economic evaluation: cost-minimization, cost-effectiveness, cost-utility, and cost-benefit. In addition, there are also descriptive studies that present economic outcomes of alternative interventions and disease, but do not directly compare health and economic outcomes. Descriptive analyses include cost-of-illness, cost identification, and cost-consequence studies. In this section, examples of evaluative and descriptive analyses in cancer prevention are provided.

*Economic Evaluations*. To promote evidence-based medical decision making, economic evaluations present the economic and health outcomes of alternative interventions. If an intervention costs more and is less effective than another intervention, the choice between the two interventions is clear. However, in many cases, the dominance of one intervention over another may depend on the relative importance of economic and health outcomes. For example, an intervention may cost more and be more effective or cost less and be less effective relative to another intervention. Economic evaluations verbally or quantitatively summarize the evidence to inform such difficult decisions.

The four types of economic evaluations (cost-minimization, cost-effectiveness, cost-utility, and cost-benefit) measure economic outcomes in monetary units, but each handles health outcomes in different ways. In cost-minimization studies, health outcomes are not measured, but assumed. For example, if two prevention interventions are known to have equivalent health outcomes, a study may examine which use the least amount of medical resources to minimize the cost to the health care system. Cost-effectiveness, cost-utility, and cost-benefit evaluations measure health outcomes, but using different units. Health outcomes in cost-effectiveness analyses are measured in natural units, such as number of life years saved. Cost-utility analyses use QALYs and cost-benefit analyses use monetary units, such as dollars. It can be difficult to translate health outcomes into QALYs or monetary units, so the typical form of economic evaluation is a cost-effectiveness analysis.

To summarize the evidence, cost-effectiveness and cost-utility analyses separate out the difference in cost and effectiveness between interventions and examine their ratio, known as an incremental cost-effectiveness ratio (ICER). This ratio measures the amount of resources required for each unit of health outcome (i.e., the amount of dollars required to

increase life expectancy by one day). The ratios can be difficult to interpret because a positive value may signify an increase in cost and an increase in effectiveness, or a decrease in cost and a decrease in effectiveness. An intervention that saves money may have the same ratio as one that requires additional resources, so it is important to look at both the ratio and budgetary implications of the choice.

Cost-effectiveness analyses of cancer screening are commonplace, particularly in the cervical cancer literature (Eddy 1990; Kulasingam and Myers 2003; Goldie et al. 2004). Brown and Garber examined three cervical screening technologies (ThinPrep, AutoPap, and Papnet) among a cohort of 20–65-year-old women from the societal perspective (Brown and Garber 1999). Outcomes of interest, including life expectancy and lifetime direct medical cost, were compared among the three technologies and between each technology and conventional Pap at various intervals. The authors found that, depending on the technology and frequency of screening, these technologies increased life expectancy by 5 h to 1.6 days and increased cost by $30–$257 (1996 U.S. dollars) relative to conventional Pap. In this case, small increases in life expectancy are related to small increases in cost. When used with triennial screening, each technology produced more life years at a lower cost relative to conventional Pap used with biennial screening. In other words, conventional Pap used with biennial screening is dominated by each technology used with triennial screening. Among the new technologies, AutoPap dominated ThinPrep, but Papnet cost $43 more and produced 0.11 additional days of life expectancy. The incremental cost-effectiveness ratio, $391 ($43/0.11) per day of life saved, suggests that if society values a day of life more than $391 then Papnet may be preferred over AutoPap. The analysis does not account for nonmedical or indirect costs and examines only life expectancy, excluding the potential burden of cancer in terms of quality of life.

Compared to cost-effectiveness analyses, cost-utility analyses have the advantage of being able to combine multiple health and clinical outcomes into QALYs. Two cost-utility analyses of cervical cancer screening have estimated health outcomes in terms of QALYs (Goldie et al. 1999; Mandelblatt et al. 2002). Goldie and colleagues assess alternative screening strategies in HIV-infected women and Mandelblatt and colleagues examine combinations of conventional Pap and HPV testing at various intervals among a longitudinal cohort of women beginning at age 20 and continuing until age 65, 75 or death. In breast cancer screening, Tosteson and colleagues compared digital and film mammography and found that using all-digital mammography is not cost-effective (Tosteson et al. 2008).

Due to their complexity in measurement and modeling, economic evaluations may be difficult to interpret and assess in terms of study quality. Their summary of the evidence is similar to a quantitative literature review, yet they often involve the prospective collection of primary data, particularly use and cost outcomes (e.g., clinical-economic trials). Guidance for the *Journal of Clinical Oncology*, put forth by Levine and colleagues (2007), identifies five key questions, which readers may ask when reviewing a study: (1) Is the question being evaluated relevant? (2) Does the study compare the appropriate alternative interventions? (3) Is the quality of the data high (e.g., economic endpoints in a clinical trial)? (4) Does the study interpret both the efficiency (i.e., cost-effectiveness) and budgetary implications? (5) Lastly, was the study sponsored by organizations without potential conflicts of interest? Like with clinical trials, a negative response to any of these questions requires greater care in the interpretation of the evidence.

Evidence on economic outcomes is not meant to dictate the choice among alternative interventions. It is only one consideration among many possible considerations. Economic evaluations are conducted to assist policy makers in their deliberation over access to cost-effective cancer prevention strategies by providing evidence on the potential impact of the alternative strategies. In the absence of evidence-based policy, cancer prevention resources may not be allocated efficiently according to the perspectives of the decision makers.

*Descriptive Studies*. Cost-of-illness studies compare economic outcomes by disease and cost-identification studies examine the difference in economic outcomes across alternative interventions. Taplin and colleagues (Taplin et al. 1995) conducted a cost-of-illness study and evaluated the direct cost of treating colon, prostate, and breast cancer. Their results suggest that the direct cost of cancer treatment increases with stage of diagnosis. Tsao and colleagues estimated that the cost of treating a patient with stage III or stage IV cutaneous melanoma is roughly 40 times the cost of treating a stage I patient (Tsao et al. 1998). Although increasing medical cost by stage may not be surprising, Ramsey and colleagues found that even after controlling for stage, direct costs were lower among persons with screen-detected versus symptom-detected colorectal cancer in the 12 months following diagnosis (Ramsey et al. 2003). The findings of these cost-of-illness studies supports the premise that primary and secondary cancer prevention may result in substantial economic benefits, potentially saving economic resources from the managed care perspective.

Cost-identification studies can improve medical decision making by dispelling perceptions of cost savings. Esser and Brunner reviewed 33 studies that examine economic outcomes of granulocyte colony-stimulating factor (G-CSF) in the prevention and treatment of chemotherapy-induced neutropenia (Esser and Brunner 2003). Contrary to conventional opinion, they found little evidence that G-CSF is cost saving as primary or secondary prophylaxis, and only minor cost savings in patients undergoing bone marrow transplant. This tertiary prevention review is particularly notable because of reports that G-CSF expenses amount to 10% of the total budget of US hospital pharmacies with limited observed clinical benefits.

Evidence from cost-identification studies may also emphasize the importance of cancer prevention as a cost containment strategy. Loeve and colleagues conducted a cost-identification study for endoscopic colorectal cancer screening and found that endoscopic colorectal cancer screening has the potential to be cost saving (Loeve et al. 2000). They stated that similar analyses of screening programs for breast and cervical cancer have not demonstrated potential cost savings under any reasonable assumptions.

Some cost-identification studies have focused on the travel and time costs of cancer prevention. O'Brien and colleagues estimated direct health service costs and the indirect cost of time off work among chemotherapy patients using patient and nurse survey data as well as administrative data from 107 participants (O'Brien et al. 1993). Houts and colleagues asked 139 patients receiving outpatient chemotherapy to keep diaries of nonmedical expenses resulting from their disease and its treatment, and documented the economic experiences of these patients (Houts et al. 1984). These small, local cost-identification studies reveal a need to better understand the nonmedical economic outcomes using a patient-centered approach. Information on out-of-pocket savings in the long-run due to cancer prevention might be useful to motivate individuals at risk, and lead them to make more informed decisions regarding their health behaviors and use of medical services.

Cost-consequence studies entail a simple tabulation of health and economic outcomes of interventions. This rare and informal type of economic analysis is like a cost-identification analysis except that it includes health outcome information. The findings of a cost-consequence study are presented without summary statements about cost-effectiveness, which distinguishes it from economic evaluations.

## 2.7 Conclusion

The purpose of this chapter was to introduce the reader to ways of quantifying the human and economic value of cancer prevention activities. The human and economic costs of cancer to individuals, families, communities, and society are substantial (Brown et al. 2001). It is imperative that personal and financial investments in cancer prevention be made; however, since healthcare resources are limited, those available must be used efficiently and equitably. To justify investments in cancer prevention, it is essential to have data about the relative costs and outcomes of prevention activities. Resources should be used for programs that produce the greatest benefit for the greatest number of people. The lack of good information about input–output relationships in health care has led to enormous variations in costs and practice patterns. The creation of more useful data and the more informed use of data currently available can enhance the public's health, patient care, and the quality of health care resource allocation decisions at many levels (e.g., individual, health plan, society).

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# The Role of Diet, Physical Activity and Body Composition in Cancer Prevention

3

C.A. Thomson, Z. Chen

## 3.1 Introduction

"Let food be thy medicine and medicine be thy food." These words were spoken by Hippocrates in 440 BC, yet today they have as much application to health and the diet-cancer link as ever. To expand on this notion, Hippocrates also stated that, "All parts of the body that have a function, if used in moderation, and exercised in labors to which each is accustomed, become healthy and well developed and age slowly; but if unused and left idle, they become liable to disease, defective in growth and age quickly." Epidemiological evidence gathered over the past 50 years continues to support the belief that over 30% of all cancers could be prevented through optimal dietary selections and a physically active lifestyle (Doll and Peto 1981). Yet over this same time span we have continued to make poor food choices, reduce our level of physical activity and experience a continuing rise in the rates of overweight and obesity to the extent that obesity has become an epidemic in the United States (US). Not surprisingly, these trends have undermined efforts to reduce cancer incidence.

This chapter will address the role of diet, physical activity and body composition in cancer prevention. In addition to summarizing the current evidence regarding associations between diet, physical activity and cancer prevention, the content will discuss the mechanistic underpinnings by which diet and physical activity can modulate the cancer process, review current guidelines for reducing cancer risk through lifestyle modification, and provide tools to support the integration of cancer preventive diets and activity patterns into clinical practice. In addition, growing evidence from intervention trials suggests a role for diet and physical activity in cancer survivorship. While this is not the primary focus of this chapter (see Chap. 18 for a discussion of issues in cancer survivorship), a review of recently completed trials is provided.

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## 3.2 Recommendations for Cancer Prevention: Body Weight, Diet, and Physical Activity

The association between diet, physical activity and cancer has been well described in the scientific literature. In terms of primary prevention, current data support the need for improved food choices and increased physical activity to prevent several of the leading cancers diagnosed in the US. Several organizations have provided guidance in terms of dietary recommendations to reduce cancer risk. Generally, the recommendations are similar across organizational groups, such as the American Cancer Society, World Cancer Research Fund (WCRF), and the Committee on Medical Aspects of Food and Nutrition Policy of the United Kingdom. The recommendations are largely based on epidemiological evidence, much of which has been supported by plausible mechanisms of biological action. In forming such policy and recommendations, these organizations often give consideration to the plausibility that individuals or populations will be able to effectively achieve and maintain the dietary pattern described. To this end, some extent of the recommendations may be more consistent with published literature than others. These reflect a positive change in intake toward an optimal level. While much of the evidence remains insufficient and further research is warranted, providing lifestyle guidelines (diet, physical activity and body weight) is prudent. These same guidelines, in a majority of the cases, also have the potential to reduce the rates of other chronic diseases beyond cancer, including cardiovascular disease, hypertension/stroke, and diabetes.

The 2007 WCRF/American Institute for Cancer Research (AICR) report which represents the most recent comprehensive and systematic review of the available evidence through 2006 suggests the following diet-related behaviors to reduce cancer risk (WCRF/AICR 2007):

- Be as lean as possible within the normal range of body weight
- Be physically active as part of every day life
- Limit consumption of energy-dense foods and avoid sugary drinks
- Eat mostly foods of plant origin
- Limit intake of red meat and avoid processed meat
- Limit alcoholic drinks
- Limit consumption of salt. Avoid mouldy grains or legumes
- Aim to meet nutritional needs through diet alone
- Mothers to breastfeed; children to be breastfed
- Cancer survivors: follow the recommendations for cancer prevention

The above advice for cancer prevention is also supported by the American Cancer Society recommendations for individual choices (Doyle 2006):

- Maintain a healthy body weight throughout life
- Adopt a physically active lifestyle
- Consume a healthy diet, with an emphasis on plant sources
- If you drink alcoholic beverages, limit consumption

## 3.3 Diet and Cancer Prevention: Review of Evidence

Historically, the majority of research indicating a cancer-protective effect for diet has largely focused on the importance of a diet high in intake of vegetables, and to some, but to a lesser extent, fruits (Riboli and Norat 2003). Evidence for other dietary components such as fiber, fat, and alcohol was less consistent, but generally indicated a probable relationship (WCRF/AICR 2007; Key et al. 2004). Table 3.1 summarizes the relationship between diet and cancer prevention including protective as well as detrimental dietary components. As shown, vegetables and fruit, as well as select bioactive food constituents within these plant foods, continue to hold promise for reducing the risk of several cancers while dietary fat/trans fats as well as animal fat and processed meats are commonly associated with increased risk. Importantly, evidence that body weight modifies cancer risk continues to mount, suggesting that the current epidemic of excess body weight/obesity in children and adults in the US will result in a significant rise in incidence of cancer over the next several decades unless dramatic efforts are undertaken to reverse this trend.

The evidence of a protective role of diet in primary cancer prevention is predominantly associated with protection against the development of solid, organ-specific tumors. Several of the most significant associations are shown for hormone-related cancers such as breast, prostate and ovarian cancer, or for cancers of the gastrointestinal tract including oral, esophageal, gastric and colorectal cancers, where there is direct contact between food constituents and the epithelial tissue. This direct contact likely contributes to mutagenic alterations in epithelial cells that either reduce or increase the risk for cellular damage, leading to the development of cancer.

Evidence over the past several decades has relied predominantly on case-control and cohort studies to describe the relationship between diet and cancer incidence. However, in recent years, prospective dietary intervention trials as well as a greater number of prospective cohort analyses have been published that suggest diet may not reduce cancer risk. Adherence to a low fat diet did not result in a reduction in risk for breast cancer among post-menopausal women enrolled in the Women's Health Initiative (WHI) Dietary Modificiation trial (Prentice et al. 2006); although a significant reduction in risk among those with the highest dietary fat intake at study entry and greatest decrease in fat intake was reported. A secondary analysis based on longer-term follow up tested the hypothesis that a low fat diet would result in significantly lower rates of other cancers. This analysis did show a statistically significant protective effect of a low fat diet related to ovarian cancer (hazard ratio, HR = 0.60, 95% confidence interval, CI: 0.38–0.96) (Prentice et al. 2007).

Two trials testing the hypothesis that increased intake of dietary fiber, with or without added fruit and vegetable intake, would modify adenoma recurrence showed that the diet interventions did not reduce the risk for adenomas of the colon (Alberts et al. 2000; Schatzkin et al. 2000). Similarly, a low-fat intervention for a period of 8.1 years did not reduce colorectal cancer risk among post-menopausal women in the WHI trial (HR = 1.08, 95% CI: 0.90–1.29) (Beresford et al. 2006). Calcium and vitamin supplementation also did not modify colorectal cancer risk in the 18,176 WHI study subjects receiving supplementation (HR = 1.08, 95% CI: 0.85–1.34) (Wactawski-Wende et al. 2006), although the

**Table 3.1** Dietary factors and associated risk for select cancers

| | Cancer type | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Lifestyle factor** | **Breast** | **Prostate** | **Colorectal** | **Lung** | **Ovary** | **Pancreas** | **Skin** | **Oral** |
| Obesity | + (postmenopause) | + | + | 0 | ± | + | 0 | + |
| Abdominal fat | + | + | + | 0 | ± | + | 0 | |
| Adult weight gain | + (postmenopause) | + | + | Unk | ± | | 0 | unk |
| Dietary factors associated with reduced cancer risk | | | | | | | | |
| Low fat (<24%) | + | + | ± | 0 | + | + | + | 0 |
| High omega 3 | + | + | + | ± | unk | unk | + | ± |
| High fiber | + | + | + | 0 | 0 | 0 | 0 | 0 |
| High vegetable | ± | ± | ± | + | | unk | unk | + |
| Carotenoid-rich | +[a] | ± Lycopene | + | + | + | | ± | + |
| Cruciferous | ±[a] | ± | + | + | | | ± | unk |
| High fruit | | | | | | + | | |
| Citrus | ± | + | ± | + | unk | | ± | + |
| Green tea | ± | ± | + | + | unk | unk | + | + |
| Lycopene | ± | + | + | + | unk | unk | + | + |
| Plant-based diet | + | + | + | + | unk | unk | unk | + |
| Selenium | ± | + | + | + | unk | unk | 0 | unk |
| Calcium | 0 | 0 | + | 0 | unk | unk | 0 | 0 |
| Vitamin D | + | + | + | unk | unk | unk | unk | unk |
| Folic acid | ± | unk | +[b] | + | unk | +unk | unk | + |
| Omega-3 fatty acids | + | + | + | unk | unk | unk | unk | unk |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dietary factors associated with increased cancer risk | | | | | | | | |
| Saturated fat | + | + | + | ± | unk | ± | unk | unk |
| Red meat | unk | + | + | + | unk | unk | unk | unk |
| Animal fat | + | + | + | + | unk | ± | 0 | 0 |
| Charred meat | + | + | + | + | 0 | unk | + | |
| Trans fatty acids | + | + | + | ± | unk | unk | unk | ± |
| Alcohol | +[c] | + | + | + | ±[c] | + | ± | + |
| Physical activity | | | | | | | | |
| Protective | + | | + | + | ± | + | unk | unk |

+An association has been demonstrated; ± – unequivocal; O – no association shown; unk – unknown association or lack thereof

[a] Intake post-diagnosis contributes to reduced risk for breast cancer recurrence

[b] Folic acid supplementation has been associated with increased risk for adenomas polyp recurrence and thus may be detrimental post-initiation

[c] While risk is increased, adequate dietary folate intake favorably modifies this risk

lack of efficacy may have been undermined by the high dietary intake of calcium reported in the study population overall. The association between Vitamin D and calcium intake and colorectal cancer also appears to be modified significantly among women who also received estrogen therapy along with supplementation (Ding et al. 2008). In fact, a recent meta-analysis of studies assessing the relationship between serum Vitamin D levels and colorectal cancer risk suggested those with the highest serum levels of 25(OH)D had a 54% lower risk of developing colorectal cancer (Gorham et al. 2007).

## 3.4 Clearing the Muddied Waters: The Need for Improved Study Designs

The above conflicting findings have contributed to considerable discussion among cancer prevention scientists in an effort to explain why evidence from population studies suggesting a protective role for diet in reducing risk of select cancers seemingly have not been upheld in randomized, controlled clinical trials. These discussions have led to renewed emphasis on study design and analysis issues to address concerns such as the recruitment of healthy volunteers, inaccurate assessment of health-related behavior, time in lifespan of healthy behavior exposure, behavior "dose," point in the carcinogenesis pathway where the behavioral intervention is being tested, and a priori hypotheses for subgroups.

First, most diet or physical activity intervention trials attract volunteers who at the time of study entry, prior to randomization to healthy behaviors or to a control group, already demonstrate habitual adoption of healthy behaviors (non-smokers, low alcohol intake, higher physical activity and lower fat intake) than the general population. It has been suggested that if trials were to enroll "average" Americans, individuals who eat few vegetables, minimal fiber and high amounts of dietary fat, a protective effect would be demonstrated in response to the dietary modification being tested. This explanation of null study results recently was demonstrated in the WHI Diet Modification trial. In this study, women consuming in the highest quartile of dietary fat at study entry demonstrated the greatest reduction in fat intake in response to the low fat diet assignment. These women also showed a significantly reduced risk for breast cancer, while analysis of the total study population was unable to show a protective effect (Prentice et al. 2006).

Second, inaccurate assessment of dietary intake and patterns of intake, either cross-sectionally or over the lifespan, continue to hinder the ability to accurately evaluate diet-cancer associations, partially because the associations are likely to be modest at best (Michels, 2005). As an example, a comparison of two separate dietary methods for quantifying dietary fat intake showed important differences in the relative risk (RR) of developing breast cancer. Food Frequency Questionnaire data resulted in a RR of 1.71 and the Seven-Day Food Record resulted in a RR of 2.09 (Freedman et al. 2006).

Third, dietary intervention trials conducted in later adulthood may be ineffective. We are attempting behavior changes that reflect an approach of "too little, too late" at a time when cells may be beyond the initiation phase.

The timing of dietary exposure in the pathogenesis of cancer may also contribute to inconsistent findings. Two key studies published in 2000 indicated that both daily consumption

of a high fiber, wheat bran cereal and daily intake of a high vegetable, fruit and fiber diet were not associated with a reduction in polyp recurrence (Alberts et al. 2000; Schatzkin et al. 2000). Although these trials were well designed, researchers continue to speculate that once a premalignant lesion is identified and the gastrointestinal tissue is "initiated," an increase in daily fiber intake will not reduce the risk for polyp recurrence. Efforts to study earlier life exposures to reduce cancer risk are increasingly being sought by funding agencies.

In addition, there is increasing evidence that there is significant variability in individual responsiveness to select dietary manipulations, much of which is the result of polymorphisms in genes associated with metabolism of nutrients or bioactive food compounds, which are thought to play a role in cancer (Hunter, 2006). Beyond genotypes, there is also strong suggestion of phenotypic variability in diet-cancer associations. For example, a recent review of diet and colorectal cancer by Jacobs and colleagues showed that gender alone is a significant modifier of significant associations (Jacobs et al. 2007). While both genders appear to reduce colorectal cancer with adequate calcium or folate intake, the data suggest that women are more likely than men to be protected by higher intake of Vitamin D and men are afforded greater risk reduction related to selenium (Jacobs et al. 2007).

There also is concern that many of the dietary intervention trials that are conducted do not sufficiently test dose-response (e.g. do not have adequate data from phase I or II studies) prior to setting an intervention target dose for the initiation of phase III randomized trials. A case in point, evaluation of the relationship between cruciferous vegetable intake and breast cancer risk suggests the data are inconsistent (Thomson et al. 2007). This inconsistency may be explained, at least in part, by the insufficient variability in amount of cruciferous vegetable intake within a study population. Further, dose of raw versus cooked vegetables is seldom assessed despite the fact that raw cruciferous vegetables are known to have greater cancer-preventive activity. Furthermore, there are limited data regarding the levels of bioactive food constituents in cruciferous or other vegetables across varieties or cultivars.

The value of large-scale behavioral interventions can be significantly increased if investigators include *a priori* hypotheses to test intervention effects in select sub-groups participating in the study. In order to do so, scientific integrity must be upheld meaning that subgroup analyses must be developed *a priori*. This can be challenging in that over the course of a study (more than 7 years in studies involving cancer endpoints), new information can become available that suggests the need for new hypotheses. For example, when the WHI was designed in late 1980s, our understanding of the role of dietary fat subtypes (i.e., trans fats, omega 3 versus omega 6 fatty acids, conjugated linoleic acid, etc) in cancer etiology was minimal, yet testing these hypotheses with the current dataset holds significant promise in advancing knowledge.

Not only has our knowledge of dietary constituents expanded over time, but we are increasingly aware of the diversity in cancer subtypes. Cancers are now described not simply by tumor size and metastatic status, but also by hormone receptor status, immunohistology, molecular expression profiles, and responsive potential to select cancer therapies. The effect of diet in relation to specific subtypes is an additional emphasis for expanded knowledge.

These realities support the need for a new approach to diet and cancer research and clinical recommendations to promote cancer prevention that considers: (1) cancer as a highly variable disease for which diet is likely to have differential effects not just based on cancer site, but also stage, molecular signature, treatment, and many other factors;

(2) an individual's habitual diet and physical activity pattern (including early life exposures), which may influence responsiveness to lifestyle interventions to reduce cancer risk; (3) genetic variability in metabolism of nutrients and biaoctive food compounds that may play an important role in responders versus non-responders; (4) dose, which should be carefully and systematically evaluated; (5) the interaction between diet and physical activity and treatment in order to evaluate potential improvements in treatment efficacy; and (6) accuracy in characterizing diet and physical activity exposures.

## 3.5 Physical Activity and Cancer Prevention

There is clear evidence that physical activity can increase lifespan and quality of life. Multiple studies support the role of physical activity in reducing cancer risk. However, the pattern of association suggests that regular moderate activity lasting more than 30 min per day is likely more protective than frequent, sustained and very intense activity (Warburton et al. 2006). A review of the available epidemiological evidence supporting a relationship between physical activity and cancer (Table 3.2) finds that the majority of evidence currently is specific to breast and colorectal cancer, although evidence for other cancer sites is evolving. The first suggestion of the protective role of physical activity dates to the early 1920s when the association between occupational physical activity and cancer prevention was first noted (Siverston and Dahlstrom 1922). Since that time, a fairly robust literature has developed that has further explored and defined this relationship. The American Cancer Society (ACS) initially published guidelines for physical activity and cancer prevention in 2002 (Byers et al. 2002). These guidelines remain essentially unchanged (Kushi et al. 2006). In the guidelines, Americans were encouraged to "adopt a physically active lifestyle," defined as:

- Adults: Engaging in at least moderate activity for ≥30 min ≥5 days of the week; ≥45 min of moderate-to-vigorous physical activity (MVPA) ≥5 days per week.
- Children and adolescents: Engaging in at least 60 min per day of MVPA at least 5 days per week.

These recommendations could be met in a variety of ways other than sports, to include leisure time physical activity (e.g. walking/hiking, swimming, resistance training); occupational physical activity (e.g. walking and lifting, manual labor) and home activities (e.g. lawn and house work).

Despite the supportive evidence, much remains unknown, including:

- Identification of the appropriate "dose" of physical activity
- Identification of the optimal timing in life for testing intervention efficacy
- Determination of the "optimal" type of physical activity
- Full understanding of mechanisms of action

It is probable that as cancer is a multi-factorial disease, there will be no "one size fits all" explanation and that to some degree variations in physical activity prevention

**Table 3.2** Association between physical activity and cancer risk, evidence from cohort studies, adapted (Thune and Furberg 2001; WCRF/AICR 2007)

| Study | Sample | Cancer site | RR (95% CI) – high vs. Low lesiure-time activity |
|---|---|---|---|
| Texas Cooper Clinic (Kampert et al. 1996) | 25,341 (M) 7,080 (F) | Overall | 0.2 (0.1–1.1) 0.4 (0.2–0.6) |
| Whitehall study (Smith et al. 2000) | 6,702 (M) | Overall | 0.8 (0.6–0.9) |
| Health Professionals' Follow-up (Giovannucci et al. 1995) | 47,273 (M) | Colon | 0.5 (0.3–0.9) |
| Nurses' Health Study (Martinez et al. 1997) | 52,875 (F) | Colon | 0.5 (0.3–0.9) |
| California Teachers Study (Mai 2007) | 120,147 | Colon | 0.75(0.57–1.00) |
| Nurses's Health Study (Wolin et al. 2007) | 79,295 (women) | Colon | 0.77 (0.58–1.01) (walking, esp. for distal tumors) |
| Swedish male Cohort (Larsson et al. 2006) | 45,906 – 496 cases | Colorectal | 0.57(0.41–0.79) |
| Harvard Alumni (Lee et al. 1991) | 17,148 (M) | Rectum | 1.7 (0.4–7.7) |
| European Prospective Investigation in Cancer (Freidenheich 2006) | 413,044 – 1,094 | Colon | 0.78 (0.59–1.03) p trend 0.04 right sided 0.65 (0.43–1.00) |
| European Prospective Investigation in Cancer (Freidenheich 2006) | 413,044 – 599 | Rectum | All non-significant |
| College Alumni (Sesso et al. 1998) | 1,566 | Breast | Total 0.7 (0.5–1.4) Pre 1.8 (0.8–4.3) Post 0.5 (0.3–0.9) |
| Nurses' Health Study (Rockhill et al. 1999) | 121,701 | Breast | 0.8 (0.7–0.9) |
| European Prospective Investigation in Cancer (Lahmann et al. 2007) | 218,169, 3,423 cases | Breast | Only household significant: Post – 0.81(0.70–0.93) Pre – 0.71(0.55–0.9) |
| Population-health screening (Thune et al. 1997) | 25,624 | Breast | Total 0.6 (0.4–1.0) Pre 0.5 (0.3–1.1) Post 0.7 (0.4–1.1) |
| Four Corners breast Cohort (Slattery 2007) | (case-1,527 Control – 1,601) > Hispanic/American Indian | Breast | No recent HRT NHW – OR – 0.60(0.36–1.02) Hispanic/American Indian-OR – 0.56(0.37–0.85) |
| Health professional Follow-up and Nurse's Health Study combined (Holick 2007) | 1,890,476 follow-up years with 706 cases | Bladder | 0.97(0.77–1.24) |

(continued)

**Table 3.2** (continued)

| Study | Sample | Cancer site | RR (95% CI) – high vs. Low lesiure-time activity |
|---|---|---|---|
| Nures's Health Study (Birmann et al. 2007) | 136,623 – 215 cases | Multiple myeloma | >7h vs <2h Male – 0.8 (0.5–1.5) Female – 0.5(0.2–1.4) |
| Swedish Twin Registry (Terry et al. 1999) | 11,659 | Endometrium | 0.2 (0.3–0.8) |
| European Prospective Investigation into Cancer (Friedenreich 2007) | 253,023 – 689 cases | Endometrial | Total – 0.88(0.61–1.27) Household/premenopausal – 0.48(0.23–0.99) |
| Swedish Mammography Cohort (Friberg 2006) | 33,723 – 199 cases | Endometrial | High leisure inactivty 1.66(1.05–2.61) |
| Iowa Women's Health Study (Mink et al. 1996) | 31,396 | Ovary | 2.1 (1.2–3.4) |
| American Cancer Society Cancer prevention Study II (Patel 2006) | 59,695 – 314 cases | Ovary | Hours of sedentary activity – > 6 per day vs. < 3 HR – 1.55(1.08–2.22) |
| Netherland's Cohort Study on Diet and Cancer (Biesma 2006) | 252 cases | Ovary | 90 min vs. < 30 min per day 0.78(0.48–1.06) |
| European Prospective Investigation Into Cancer (EPIC) (Berrington 2006) | 438,405, 324 cases | Pancreatic | 0.82(0.50–1.35) |
| Harvard Alumni (Lee and Paffenbarger 1994) | 17,607 | Prostate | 0.6 (0.2–1.4) |
| Physicians' Health Study (Liu et al. 2000) | 22,071 | Prostate | 1.1 (0.9–1.4) |
| NHANES I (Clarke and Whittemore 2000) | 5,377 | Prostate | C 1.7 (0.8–2.3) AA 3.7 (1.7–8.4) |
| Norweigen Cancer registry (Nilsen 2006) | 29,110–957 cases | Prostate | High versus no physical activity 0.64(0.43–0.95)advanced disease |
| Vitamins and Lifestyle (VITAL) cohort (Littman 2006) | 34.757 – 583 cases | Prostate | >10.5 MET-h per week vs no activity-HR −0.69(0.46–1.0) |
| Norway General Population (Thune and Lund 1994) | 53,242 | Testicle | 2.0 (0.6–6.9) |
| Harvard Alumni (Lee et al. 1999) | 13,905 | Lung | 0.4 (0.4–1.0) |
| Iowa Women's Health Study (Sinner 2006) | 36,929 | Lung | 0.77(0.64–0.92) |
| European Prospective Investigation into Cancer (EPIC) (Steindorf 2006) | 416,277 – 1083 cases | Lung | Only for sports in males 0.71(0.50–0.98) Cycling in females 0.73(0.54–0.99) |

*RR* Relative risk; *CI* Confidence Interval; *LPA* Leisure time physical activity; *Pre* Premenopausal; *Post* Postmenopausal; *C* Caucasian; *AA* African American

recommendations will exist. Nonetheless, the above-stated guidelines are a good starting point in moving individuals to a more active health-promoting lifestyle.

Note that the associations identified in large epidemiological trials account for only small reductions in risk; however, the potential additive effects of several lifestyle modifications could be considerable. In addition, these are modifications that are achievable, cost-effective and have no or minimal side effects. Thus, pursuit of such changes in lifestyle is an appropriate strategy to reduce cancer risk (Sass 2002).

## 3.6 Body Weight and Body Composition and Cancer Prevention

Body weight and composition have also been identified as modifiable risk factors for cancer. In fact, recent recommendations for cancer prevention have placed increased emphasis on the importance of attaining and maintaining a healthy body weight. Table 3.3 outlines the current associations between overweight/obese status and cancer risk. Generally, obesity has been identified as a risk factor for several solid tumors as well as hormone-related cancers. A pooling project analysis assessing the relationship between body weight and colorectal cancer confirms earlier work that increased body mass index (BMI) is positively associated with risk, especially among males (Jacobs et al. 2007). Beyond hormone-related and solid tumors, a recent analysis from a combined dataset of Nurse's Health Study and the Health Professionals Follow-up Study suggests that being overweight or obesity (BMI > 25) may also increase the risk for multiple myeloma, particularly among men (Birmann et al. 2007). The 2007 WCRF/AICR report suggested that the evidence was convincing or probable for an increased risk of esophageal, pancreatic, gallbladder, colorectal, post-menopausal breast, endometrial, and kidney cancers (WCFR/AICR, 2007). Abdominal fat has also been assessed in select populations and indicates an increased risk for pancreatic, colorectal (male and female), post-menopausal breast, and endometrial cancers (WCRF/AICR 2007).

Importantly, the relationship between obesity and cancer risk is not straightforward, in that numerous confounders or effect modifiers have been identified that may influence

| Cancer site | Percentage of cancers associated with obese status |
|---|---|
| Breast (post-menopausal) | 9 |
| Colorectal | 11 |
| Endometrial | 39 |
| Esophagus | 12 |
| Kidney | 25 |
| TOTAL | 5 |

**Table 3.3** Role of body weight and body composition in cancer prevention, adapted from IARC/WHO (IARC 2002)

these complicated associations. As an example, gender-specific associations have also been shown to modify risk. A meta-analysis of studies evaluating the role of obesity in relation to rectal cancer risk suggested that risk was significantly increased among males, but not females (Larsson and Wolk, 2007). Similarly, data from the European Prospective Investigation into Cancer (EPIC) showed an increased risk for colon cancer related to BMI and body weight among males, but not females (Pischon et al. 2006). Interestingly, both genders were at elevated risk for this disease related to greater waist circumference or waist-hip ratio, suggesting that clinically we need to assess these parameters routinely rather than solely relying on body weight to determine the need for weight control interventions. In testing associations between obesity and breast cancer, it is clear that menopausal status is a major risk modifier. Further, an analysis from the EPIC study has demonstrated that while obesity modifies post-menopausal risk among women not taking hormone replacement, when hormone replacement is occurring the risk associated with obese status is nullified (Lahmann et al. 2004).

Weight change over the lifespan has also been identified as a risk factor for select cancer, although the majority of research has focused solely on breast cancer risk. The EPIC study showed an 8% increase in post-menopausal breast cancer risk among non-users of hormone replacement therapy for every 5 kg of weight gain over adulthood (Lahmann et al. 2005). These results were supported by data from the Nurse's Health Study cohort, an analysis of which that also suggested a 57% reduction in post-menopausal breast cancer risk among women who lost more than 10 kg of body weight in adulthood and who maintained this reduced body weight (Eliassen et al. 2006). The Iowa's Women's Health Study also tested these associations and provided additional evidence that weight loss even after menopause was beneficial to reducing breast cancer risk (Harvie, et al. 2005). This same cohort has been used to demonstrate a protective effect of intentional weight loss during adulthood among overweight women (e.g. women who lost more than 20 pounds, resulting in normal weight status that was sustained) in terms of reduced risk for breast, colon, and endometrial cancers (Parker and Folsom 2003). Adult weight gain of greater than 60 pounds after age 18 years has also been associated with poorer prognostic breast tumor indicators including stage, grade, estrogen receptor and progestin receptor negative (ER-/PR-) tumors (Feigelson et al. 2006). These data while generally reflective of breast cancer risk are likely to be repeated in other obesity-related cancers as additional analyses are completed over the coming years.

The association between obesity, weight gain and tumor characteristics may partially explain why, in addition to the increased cancer risk of being overweight/obesity, it also appears to increase mortality risk associated with select cancers (Calle et al. 2003). This association was initially reported in the American Cancer Society (ACS) cohort in the late 1970s and showed a "J-shaped" association between body weight and cancer. This "J-shape" association between body weight and cancer suggest that extremes in body mass index (BMI) at both the lower and upper ends of the range are associated with increased mortality risk (Banegas et al. 2003) and were further substantiated in 2007 in the Million Women study in UK (Reeves et al. 2007). Of relevance to clinical practice, Flegal and colleagues demonstrated that mortality was significantly increased among obese (BMI > 30 $kg/m^2$) cancer patients *only if* they had been previously diagnosed with a cancer known to be obesity-related (Flegal et al. 2007). Finally, there is some evidence to suggest that overweight/obese status also increases the risk for secondary cancers among subjects

previously treated for cancer. In one analysis, the National Health Insurance Corporation Study, the relative risk for second primary tumors among overweight men was elevated for colorectal (RR = 3.45) and for genitourinary cancers (RR = 3.61) (Park et al. 2007), suggesting that males with these diagnoses should receive significant support to lower body weight as part of their cancer treatment plan.

The above evidence suggests that body weight is a modifiable risk factor in terms of the primary prevention of cancer as well as a prognostic indicator for select cancers. Therefore, prevention of undesirable body weight status (as well as prevention of increases in waist circumference and waist-to-hip ratio) should be considered in the first line of defense against the development of cancer. In fact, there are known periods in life when people are more vulnerable and undesirable weight gain is more common. These include:

- Infancy (promotion of breastfeeding and avoidance of the push for "catch-up" growth among low birth weight infants)
- Early childhood (related to structured feedings versus eating to appetite as well as restricting access to energy-dense foods)
- Adolescence/pre-pubertal
- Entry into the work force
- College entry
- Marriage
- Childbirth
- Menopause
- Retirement
- Stressful life events, such as death of a significant other, divorce, illness
- Activity-limiting illness (including cancer and even routine surgeries that restrict activity post-operatively and significantly increase loss of lean tissue mass)
- Select medication use (i.e., oral contraceptives, post-menopausal hormone therapy, anti-depressants, insulin, etc.)

Not only should research focus on these vulnerable time periods in life, clinicians should also be acutely aware of circumstantial changes in their patients' lives that may place them at risk of undesirable weight gain or shifts in body fat-to-lean mass ratio. To this end, clinicians should not limit assessment to monitoring for changes in body weight over time, but should also proactively instruct patients regarding weight gain prevention during these vulnerable life circumstances.

## 3.7 Lifestyle and Cancer Survivorship

As survival rates for cancer have steadily increased (current estimates suggest there are over 9.8 million cancer survivors in the US alone) (Jemal 2007), there has been substantial interest generated related to the role of lifestyle modification in preventing cancer recurrence and enhancing the quality of life (QOL) for cancer survivors, both during and after

treatment (Courneya et al. 2003). Much of the literature surrounding cancer survivorship is presented in Chap. 19. Here we specifically focus on the role of diet and physical activity. Historically, clinicians and researchers have relied on the primary prevention model to direct patients regarding optimal diet and physical activity patterns to reduce cancer recurrence risk. This approach continues to be promoted among cancer organizations today (Doyle et al. 2006).

Cancer survivors are a particularly appropriate population for assessing the role of lifestyle change in reducing cancer risk. This is because cancer survivors demonstrate a higher cancer incident rate than the general population (allowing for reduced study sample size and time for testing hypotheses prospectively). A survey of health behaviors of over 7,000 cancer survivors using the National Health Interview Survey showed that smoking and alcohol intake were similar for cancer survivors and controls, although survivors were 9% more likely to meet physical activity recommendations (Bellizzi et al. 2005). Female breast cancer survivors frequently report and demonstrate high levels of motivation to improve diet-related behaviors on the personal level after cancer diagnosis (Thomson et al. 2002). However, physical activity levels measured at time of diagnosis and 6 months post-diagnosis appear to drop in most women while increasing in others (Andrykowski et al. 2007). This suggests that education may be needed to promote a physically active lifestyle post-diagnosis. There is also evidence that adolescents experience a significant reduction in physical activity following cancer treatment, which is sustained longterm in approximately 14% of youth, possibly related to on-going fatigue (Keats et al. 2006).

## 3.8 Body Weight and Cancer Survival

As discussed above, body weight is a primary risk factor for select cancers and likely influences survival in obesity-related cancers. However, one should keep in mind that the magnitude and direction of the association between body weight and cancer survival varies depending on the type of cancer. For example, increased body weight and body fat may negatively affect the prognosis of breast cancer, while survivorship in lung cancer patients is improved among those with stable weight or even weight gain. Historically, weight gain was a common problem among breast cancer patients who receive adjuvant chemotherapy (Dixon et al. 1978; Heasman et al. 1985; Ganz et al. 1987; Demark-Wahnefried et al. 1993; Demark-Wahnefried et al. 1997; Goodwin et al. 1999); however, the magnitude of the problem has been reduced over time (Saquib et al. 2007).

Using breast cancer as an example to evaluate body fat distribution and survival, it is clear that inconsistent results persist. A study in the Netherlands found no association between central fat distribution, measured by the ratio of subscapular and triceps skinfold thickness, and survival time in breast cancer patients, after stratification by axillary node status, estrogen receptor status, and method of cancer detection (den Tonkelaar et al. 1995). Kumar and colleagues suggested that among breast cancer patients, android body fat distribution, as indicated by a higher suprailiac to thigh ratio, was a statistically significant ($p < 0.0001$) predictor of poorer 10-year survivorship (Kumar et al. 2000). In contrast,

Schapira and colleagues reported that peri-menopausal and post-menopausal women with greater upper body fat distribution appeared to be a subset of women with a more favorable prognosis, as measured by less lymph node involvement, smaller tumors, and higher levels of ER in their tumors (Schapira et al. 1991).

Healthy behaviors may also influence the association between obesity and cancer risk. In an analysis of diet and physical activity habits among over 1,500 breast cancer survivors, Pierce and colleagues showed a significant reduction in breast cancer recurrence among those who exercised at least 30 min a day, 6 days per week, and who consumed greater than five servings of vegetables and fruits daily (Pierce et al. 2007). This finding was consistent even among survivors with BMI over 25 $kg/m^2$.

Clearly, more studies are needed to understand the impact of body fat distribution on cancer prognosis among breast (and other) cancer survivors. It is concerning that in 2008 there is a paucity of intervention trials with large sample sizes being conducted among cancer survivors in the US to promote weight control as a means to reduce cancer recurrence or mortality for which hard disease endpoints are being collected. Several small trials to test the efficacy of study interventions in terms of weight and body composition outcomes as well as clinical parameters of health status (e.g. lipid levels, fasting insulin, insulin-like growth factors, oxidative stress) have been and continue to be conducted.

## 3.9 Survivorship and Diet

Outcomes have recently been published for two large, longitudinal diet intervention trials conducted among breast cancer survivors to test hypotheses related to post-therapy diet modification and risk for disease recurrence and survival. The first, the Women's Intervention Nutrition Study (WINS), conducted between 1993 and 2003, enrolled 2,437 post-menopausal breast cancer survivors across 39 clinical sites throughout the US (Chlebowski et al. 2006). Women were randomized, within one year of completion of primary treatment of breast cancer, to either a 15% energy from fat diet (40% of subjects) or usual diet (60% of subjects) for an average of seven years. Interim results showed a statistically significant 42% reduction in cancer recurrence among women diagnosed with ER-negative tumors randomized to the low-fat diet as compared to the usual diet group. These data suggest that women diagnosed with ER-negative tumors may benefit from dietary counseling to reduce dietary fat to less than 15% of total energy intake. There was a small (6 pounds over 7 years) reduction in body weight associated with this protective association. This finding will need to be explored further in future studies.

A second study, the Women's Healthy Eating and Living Study (WHEL) also targeted breast cancer survivors for participation in a 7.3-year dietary intervention trial testing the hypothesis that a plant-based diet (20% energy as fat, 5 vegetables and 3 fruits daily, 16 ounce fresh vegetable juice daily, and more than 30 g fiber daily) would significantly lower breast cancer recurrence among the 3,088 women enrolled between 1994 and 2000 (Pierce et al. 2002). In this study, despite significant and sustained changes toward a healthier eating pattern, breast cancer events were not reduced significantly among women assigned to

the plant-based diet as compared to the control diet (Pierce et al. 2007). One explanation for the null findings is the fact that study subjects reported relatively healthy eating habits at the time of study enrollment and also reported having improved the quality of their food selections shortly after breast cancer diagnosis and, on average, 2.3 years prior to study enrollment (Thomson et al. 2002). This explanation is further supported by an analysis of recurrence rates in the control subjects published by Rock and colleagues that showed a significant reduction in breast cancer recurrence among WHEL women who demonstrated the highest levels of plasma carotenoids at the time of study entry (Rock et al. 2005).

While these studies may appear to provide conflicting results, several differences in study design likely contributed to differential diet-disease associations. First, the WINS study resulted in a significant decrease in dietary fat intake that brought intake to a level suggested to modify insulin or inflammatory markers important risk factor markers for breast cancer. The extent of reduction in dietary fat (mean intake 28.6% energy) in the WHEL study was not as great as what was achieved in the WINS trial. Second, there is the possibility that the clinical timing of dietary intervention is of importance to efficacy. In the WINS trial the intervention was initiated within one year post-surgery, and in the WHEL study, women were recruited between one and 4 years post-diagnosis and earlier analysis of self-reported dietary change showed that the WHEL study participants had already made significant changes in dietary intake (especially in terms of fat reduction and more fruit and vegetable intake) prior to enrollment in the study. Third, chemotherapy was an eligibility criteria for WINS participation (and a greater number received CMF than AC), while approximately 30% received no chemotherapy in the WHEL study sample. WINS was also restricted to post-menopausal disease, WHEL was not.

So while two large randomized dietary intervention trials may appear to suggest opposing results, close evaluation indicates that significant differences in study design, target populations and clinical treatment as well as baseline dietary patterns at the time of study enrollment likely explain the variable results. Of importance, breast cancer survivors are in need of guidance on the issue of lifestyle change post-diagnosis. Based on these trials as well as related sub-group analyses from the same populations as well as other epidemiological evidence, the current recommendations would be:

1. Reduce dietary fat intake to 15–20% energy from fat daily (post-menopausal with ER- tumors)
   a. *WINS trial approach: body weight in pounds/6 (i.e. 180 pounds/6 = 30 g dietary fat daily)*
2. Maintain an intake of vegetables and fruit of 5–7 + servings per day
3. Be physically active (>30 min moderate activity daily)
4. BMI should be below 30 kg/m$^2$ and optimally between 19 and 25; slight weight loss (1–3 lb per year) may be beneficial in reducing recurrence risk

Finally, an analytical cohort of dietary patterns (Western versus Prudent) reported among stage III colorectal cancer patients has been published. After a mean 5.3 years of follow-up, patients who reported consuming a Western type diet that was higher in fat and lower in vegetables, fruit and fiber as compared to the Prudent diet, demonstrated a significantly poorer survival (Meyerhardt et al. 2007). The adjusted HR was 3.25 for disease-free survival, 2.85 for recurrence-free survival and 2.32 for overall survival.

The combined findings of these trials, while limited, support the proposition that cancer survivors should adopt healthy behaviors for diet, physical activity and weight control as soon as possible after cancer diagnosis in an effort to reduce their risk for disease recurrence and improve their overall survival.

## 3.10 Physical Activity and Cancer Survivorship

The role of physical activity in reducing the risk for cancer recurrence has received minimal scientific attention to date. However, with increasing survival, this is a fast growing research area. A comprehensive review of the literature in 2004 identified 43 studies – 21 randomized controlled trials and 22 quasi-experimental studies (Irwin and Ainsworth, 2004). The majority were feasibility studies that looked primarily at psychosocial outcomes rather than physiological measures. Overall, evidence suggests that among patients currently undergoing therapy and/or previously treated for cancer, increased energy expenditure in the form of regular physical activity provides benefits in biopsychocial and physiological measures. These factors have been most studied in women with breast cancer, and to a lesser degree in other cancers (Irwin and Ainsworth, 2004).

In another review of this literature, 19 intervention trials and nine observational studies have been reviewed in women either undergoing treatment for breast cancer or post-therapy. Statistically significant results have been demonstrated in a number of objective and subjective outcome measures (Courneya et al. 2003). These have included exercise and functional capacity, body weight and composition, flexibility, nausea, physical well-being, functional well-being, mood states, anxiety and depression, satisfaction with life, and overall quality of life. These trials, as reviewed by Courneya et al. (2003), have used a variety of exercise interventions (e.g. self-directed or home-based versus supervised exercise) of variable study duration. Many have made use of the traditional "exercise prescription" guidelines with respect to frequency, intensity, type and tempo with only a few studies looking at resistance training.

Cardiovascular fitness and QOL were assessed in 53 post-menopausal women who had received treatment for breast cancer. They were randomly assigned to an exercise group or control. The intervention consisted of training on a bicycle ergometer three times per week for 15 weeks. Primary outcome measures included changes in $VO_{2max}$ and QOL, as measured by the Functional Assessment of Cancer Therapy-Breast subscale. Peak oxygen consumption increased by 0.24 L/min in the exercise group, whereas it decreased by 0.05 L/min in the control group (mean difference, 0.29 L/min; 95% confidence interval [CI], 0.18–0.40; $p < 0.001$). Overall QOL increased by 9.1 points in the exercise group compared with 0.3 points in the control group (mean difference, 8.8 points; 95% CI, 3.6 to 14.0; $p = 0.001$). Pearson correlations indicated that change in peak oxygen consumption correlated with change in overall QOL ($r = 0.45$; $p < 0.01$). The researchers concluded that this exercise intervention positively affected cardiovascular fitness and QOL in post-menopausal women who had undergone treatment for breast cancer (Courneya et al. 2003).

One trial evaluated the effects of structured exercise on physical functioning and other dimensions of health-related QOL in women with stages I and II breast cancer (Segal et al. 2001). One hundred twenty-three women with stage I or II breast cancer were randomly allocated to one of three intervention groups: usual care (control group), self-directed exercise, or supervised exercise. Quality of life, aerobic capacity, and body weight measures were repeated at 26 weeks. The primary outcome measure was the change in the Short Form-36 physical functioning scale between baseline and 26 weeks. Findings demonstrated a decrease in physical functioning in the control group by 4.1 points, whereas it increased by 5.7 points and 2.2 points in the self-directed and supervised exercise groups, respectively ($p = 0.04$). Post-hoc analysis demonstrated a significant and clinically important difference between the self-directed and control groups (9.8 points; $p = 0.01$) and a more modest difference between the supervised and control groups (6.3 points; $p = 0.09$). No significant differences between groups were observed for changes in quality of life scores. In a secondary analysis of participants stratified by type of adjuvant therapy, supervised exercise improved aerobic capacity (+3.5 mL/kg/min; $p = 0.01$) and reduced body weight (−4.8 kg; $p < 0.05$) compared with usual care, but only in participants not receiving chemotherapy. The researchers concluded that exercise can lessen some of the negative side effects of breast cancer treatment. Self-directed exercise was seen as an effective method to improve physical functioning as compared to usual care. In participants not receiving chemotherapy, supervised exercise could increase aerobic capacity and reduce body weight compared with usual care. Whereas methodologies have often differed, sample sizes have been small, and research vigor has been variable, thereby limiting interpretation of findings, the cumulative evidence supports the safety and benefits of physical activity for women who have breast cancer.

Researchers have also evaluated physical activity in men undergoing androgen deprivation therapy for prostate cancer (Segal et al. 2003). This treatment often causes fatigue, functional decline, increased body fatness, and loss of lean body tissue, side-effects that may negatively affect health-related quality of life. They hypothesized that resistance exercise would counter some of these side-effects by reducing fatigue, elevating mood, building muscle mass, and reducing body fat. They recruited 155 men with prostate cancer scheduled to receive androgen deprivation therapy for at least 3 months after recruitment. Volunteers were randomly assigned to an intervention group that participated in a resistance exercise program three times per week for 12 weeks (82 men) or to a waiting list control group (73 men). The primary outcome measures were fatigue and disease-specific quality of life as assessed by self-reported questionnaires; secondary outcome measures were muscular fitness and body composition. Men assigned to resistance exercise had less interference from fatigue on activities of daily living ($p = 0.002$) and higher quality of life ($p = 0.001$) than men in the control group. Men in the intervention group demonstrated higher levels of upper body ($p = 0.009$) and lower body ($p < 0.001$) muscular fitness than men in the control group. The 12-week resistance exercise intervention did not improve body composition as measured by changes in body weight, body mass index, waist circumference, or subcutaneous skinfolds. They concluded that resistance training could decrease fatigue, improve QOL, and enhance musculoskeletal fitness in men with prostate cancer receiving androgen deprivation therapy (Segal et al. 2003).

One hundred and two survivors of colorectal cancer were randomly assigned to an exercise group versus control in another research trial (Courneya et al. 2003). The exercise group was asked to perform moderate intensity exercise 3–5 times per week for 20–30 min for 16 weeks. The control group was instructed not to enroll in a structured exercise program. The primary outcome measure was change in QOL as measured by the Functional Assessment of Cancer Therapy-Colorectal (FACT-C) scale. Although adherence in the exercise group was good (75.8%) significant contamination in the control group occurred (51.6%). Intention-to-treat analysis revealed no significant differences between groups for change in the FACT-C (mean difference −1.3; 95% CI: −7.8–5.1; $p = 0.679$). In an 'on-treatment' ancillary analysis, comparison was performed of participants who decreased versus increased their cardiovascular fitness. This analysis revealed significant differences in favor of the increased fitness group for the FACT-C (mean difference 6.5; 95% CI: 0.4–12.6; $p = 0.038$). The researchers concluded that increased cardiovascular fitness was associated with improvements in QOL in colorectal cancer survivors (Courneya et al. 2003).

A meta-analysis of physical activity interventions conducted among cancer survivors conducted between 1970 and 2004 (n = 30 studies) suggests that (1) study sample sizes are small to date, (2)attrition rates are acceptable and not significantly different between intervention and control groups, (3) on average interventions lasted 10 weeks, with only one trial evaluating outcomes at one year (Conn et al. 2006). Overall the data support the role of exercise in reducing measured outcomes such as body weight and fatigue while improving mood, quality of life and physical function. In contrast, a recent intervention trial, the Active for Life After Cancer randomized trial was not able to demonstrate improvements in QOL or physical function among 134 prostate cancer patients (Carmack et al. 2006).

It is important to recognize that during the various phases of cancer treatment and post-treatment, people often experience significant limitations in their functional capacity. Fatigue, diminished exercise capacity, and decreased strength often exist. The decision how best to incorporate physical activity necessarily requires individualization based upon pre-existing exercise levels, current physical status, and goals and expectations. For those with low pre-existing physical activity levels, simple stretching or a few minutes of walking performed regularly may be all that is tolerated. For those with more active backgrounds, maintenance of levels may be desirable. Physical activity levels may be increased as physical abilities are enhanced. With the paucity of available data, it is probable that the guidelines published by the American Cancer Society for prevention are appropriate for prevention of recurrence (Byers et al. 2002).

## 3.11 Optimizing Bone Health

Bone metabolism can be disrupted by metastatic bone disease that often develops as a result of many interactions between tumor cells and bone cells. Cancer patients with bone metastases may experience bone pain, fractures, hypercalcemia and spinal cord compression. Given the long survival time after bone metastasis in certain types of cancer, these

skeletal complications may have profound adverse effects on cancer survivors' quality of life. There is some evidence that bisphosphonates that are used to promote bone integrity clinically may not only reduce the symptoms and complications of cancer-related bone metastases, but may also inhibit the development of bone metastases (Coleman, 2001).

Low bone density, deterioration of bone microstructure, and increased risk of fracture are characteristics of osteoporosis. Poor nutrition, reduced sex hormones levels, prolonged pharmacological intervention, disease, and low physical activity level or decreased mobility are all risk factors for osteoporosis. As a result of cancer prognosis and treatment, patients with cancer are often predisposed to low bone mineral density (BMD) and increased risk for osteoporotic fractures in later life. Cancer treatments may increase the risk for developing osteoporosis through a variety of biological mechanisms, including treatment-induced hypogonadism, disturbances of thyroid and growth hormones, hormone-independent effects of chemotherapy on bone cells, and nutrient malabsorption after gastrectomy (Pfeilschifter and Diel 2000).

Studies have shown that young women who experience premature menopause following chemotherapy for breast cancer demonstrate excess bone loss and a higher risk for early development of osteoporosis (Headley et al. 1998; Shapiro et al. 2001; Vehmanen et al. 2001). The rate of bone loss among post-menopausal breast cancer patients is less well characterized. However, a recent study found that regardless of whether the breast cancer diagnosis is before or after menopause, women with a history of breast cancer often have an increased risk for bone fractures after age 50 (Chen et al. 2005).

Anti-resorptive agents may reduce the bone loss associated with chemotherapy-induced ovarian failure in premenopausal women (Saarto et al. 1997). It has been reported that adjuvant clodronate treatment significantly reduced chemotherapy-induced bone loss (Vehmanen et al. 2001). However, clodronate may have limited effects in preventing bone loss among post-menopausal breast cancer patients after the termination of hormone replacement therapy (HRT). In a randomized control trial among post-menopausal breast cancer patients, women who had recently discontinued HRT experienced more rapid bone loss than HRT non-users. Neither three-year anti-estrogen therapy alone nor anti-estrogen together with clodronate could totally prevent the bone loss related to HRT withdrawal, even though clodronate seemed to retard it (Saarto et al. 1997). Recent findings have suggested that anti-resorptive agents, such as risedronate once weekly, may effectively prevent bone loss in breast cancer survivors who were treated with chromtherapy (Greenspan and Bhattacharya 2007).

Prostate cancer is the most common malignancy among American men. Since prostate cancer is testosterone dependent, androgen deprivation therapy (ADT) with GnRH agonists (GnRH-a) is often used in treating prostate cancer, which renders these men hypogonadal. Testosterone plays an important role in maintaining bone as well as skeletal muscle mass; ADT-induced hypogonadism potentially has a significant impact on body composition. Smith reviewed studies on bone loss and osteoporosis-associated ADT among men with prostate cancer (Smith, 2002). The cumulative evidence indicates that prostate cancer patients without apparent bone metastases are at increased risks of osteoporosis and obesity, as well as low lean and soft tissue mass, after ADT (Stoch et al. 2001; Basaria et al. 2002; Berruti et al. 2002; Chen et al. 2002b). Bisphosphonates reduce the loss of bone density among prostate cancer patients after ADT (Smith et al. 2001). Recent studies have

provided further evidence of the efficacy of different types of bisphosphonates for preventing and slowing down bone loss in men treated with ADT. (Ryan et al. 2007; Rodrigues et al. 2007). However, in spite of the advancement in understanding treatment effects on ADT-reduced bone loss, there is a lack of significant improvement in osteoporosis management for this patient population. A new study showed that only a small percent of men treated with ADT for prostate cancer received osteoporosis screening, prevention or treatment. There is a significant need to identify optimal strategies for screening, prevention, and treatment of ADT-reduced bone loss in prostate cancer patients. (Yee et al. 2007).

The increasing incidence of childhood cancers and improvements in treatment for childhood malignancies have resulted in a growing number of childhood cancer survivors in the US. The improved survival of children with malignant diseases is in part due to the application of intensive, multi-modality therapies that include radiotherapy, surgery, glucocorticoids, and cytotoxic agents. However, such interventions have the potential to induce complex hormonal, metabolic, and nutritional effects that may interfere with longitudinal bone growth and skeletal mass acquisition during the critical growth phases of childhood and adolescence. In a review paper, van Leeuwen and colleagues (van Leeuwen et al. 2000) concluded that many factors might simultaneously or independently affect final height and BMD in adults who survived childhood cancers. Multi-agent chemotherapy, corticosteroids, malignancy itself, cranial radiotherapy, and physical inactivity may reduce long bone growth resulting in short body height. These factors may also cause low peak bone mass, which may lead to long-term consequences including increased risk for bone fractures in later life.

Total and near-total thyroidectomy, with or without radioiodine ablative therapy, are in the mainstay of current treatments for well-differentiated thyroid cancer. Supra-physiologic amounts of oral thyroxine are also administered to suppress serum thyroid stimulating hormone (TSH) to below detectable levels. It is well known that overt clinical hyperthyroidism may increase bone turnover and decrease BMD, but the effect of suppressive thyroxine treatment on BMD is less well known. After reviewing current evidence on bone density among thyroid cancer patients who were treated with suppressive thyroxine, Quan and colleagues (Quan et al. 2002) concluded that there is no significant change in BMD for premenopausal women or in men after diagnosis and treatment for thyroid cancer; however, the findings for post-menopausal women are controversial and remain unclear.

Gastric cancer is one of the most common causes of cancer death. The only treatment that leads to cures in some patients is surgery. Liedman has reviewed the impact of gastrectomy on food intake, body composition, and bone metabolism (Liedman 1999). Previous studies have suggested that substantial weight loss, amounting to about 10% of preoperative weight, occurred during the early post-operative period, including a decrease in lean soft tissue. To study the changes in body composition and BMD after gastrectomy, Liedman and colleagues (Liedman et al. 2000) conducted a small longitudinal study among 22 gastric cancer patients who were long-term survivors after total gastrectomy (mean of 8 years). Whole body dual x-ray absorptiometry (DXA) scans were performed at a mean of 5 and 8 years after the surgical treatment. The results showed that patients lost an average of 3.2 kg of body weight ($p < 0.006$) with a corresponding loss of lean body mass ($p < 0.0001$). However, there was no difference in bone density from values seen in age- and sex-matched controls. There was a slight elevation of osteocalcin levels and a

minor increase in parathyroid hormone levels. The impact of total gastrectomy on calcium homeostasis and BMD seems to be marginal in this study. However, the authors argued that the observed close relationship between BMD and body weight suggested the pivotal importance of maintaining lean body weight after gastrectomy.

There is very limited information on bone density among cervical cancer and ovarian cancer patients. A small case-control study in 40 cervical cancer patients treated with radiotherapy and 40 matched controls found no significant difference in the BMD between the two groups and no significant change in BMD 1 to 7 years after therapy in the patient group (Chen et al. 2002a). In another small study, fifteen women (mean age 38.2 ± 7.8 years; range 30–46 years) with ovarian cancer who had been treated with chemotherapy for six cycles every four weeks following surgical cytoreduction were measured for bone loss. A significant bone loss was observed ($p < 0.001$), and baseline lean mass predicted bone loss with anti-cancer chemotherapy (Douchi et al. 1997).

It is fundamental that health professionals and cancer patients learn about the risk of osteoporosis associated with both cancer prognosis and treatment and to take an active role in preventing, treating and managing osteoporosis. Preventive measures should be recommended to all patients. Risk assessments, including BMD testing for osteoporosis, should be performed, and treatments should be initiated for people with low bone density or at high risk of bone fracture. Although specific guidelines and consensus on the treatment and prevention of osteoporosis among cancer survivors are lacking, research evidence suggests that both non-pharmacologic approaches and some pharmacologic approaches to prevent and treat osteoporosis among the general population may be applicable to cancer populations (Mincey et al. 2000; Pfeilschifter and Diel 2000).

Pharmacologic approaches include anti-resorptive therapy and anabolic therapy. Most of the bone-active agents currently available in the US act by inhibiting bone resorption. Estrogens, selective estrogen receptor modulators (SERMs), bisphosphonates, calcitonin, calcium and vitamin D all have anti-resorptive properties. However, estrogen therapy is neither an option for cancer survivors nor recommended for prevention or treatment of osteoporosis for women without vasomotor symptoms. There are several anabolic agents that have the ability to increase bone formation. These include parathyroid hormone, fluoride, growth hormone, insulin-like growth factor-1, androgens, tibolone, strontium and statins. Since many of these agents may increase the risk of malignancy, they are not good candidates for osteoporosis therapy among cancer patients or those at risk of cancer.

Falls are responsible for over 90% of hip fractures. Nutrition, exercise and fall prevention are important non-pharmacologic approaches for fracture risk reduction. Adequate calcium and vitamin D intake are necessary for bone health. Vitamin K, caffeine, soy, and high protein and sodium diet may also affect bone density, but their relationship to osteoporosis has not been well established. Exercise provides an approximate twofold benefit for osteoporosis risk reduction, including significant improvements (or maintenance) in bone density as well as reduced risk of falls. In a 2004 report on osteoporosis by the US Surgeon General, lifestyle approaches to promote bone health are supported by a rich body of research evidence (DHHS 2004). An analysis using the data from the Women's Health Initiative has indicated that the number of falls increased significantly after breast cancer or other invasive cancer diagnosis. However, the increased number of falls did not seem to be the major cause of the increased fracture risk in these post-menopausal cancer patients

(Chen et al. 2008). Future studies are needed to better understand the attribution of falls and bone loss as well as other factors, such as changes in bone geometric structures, to the higher fracture risk observed in post-menopausal cancer populations.

## 3.12 Mechanisms of Carcinogenesis Modified by Lifestyle Factors

Although there are literally dozens of biological mechanisms by which diet and physical activity protect against the development of cancer, a few have been of particular biological relevance. These include insulin resistance, modulation of immunity, including the inflammatory response, and DNA damage-repair. Each of these are described and discussed in more detail below (Fig. 1).

*Insulin Resistance*. Insulin resistance has been described as the dysregulation of insulin response to elevations in blood glucose levels. Among individuals demonstrating insulin resistance, even moderate intake of low fiber, high carbohydrate food items (high glycemic foods) can result in significant elevations of plasma insulin levels. Insulin is well known to be growth promoting, and thus, increased plasma insulin levels in the presence of pre-cancerous or cancer cells are thought to provide the necessary microenvironmental stimulus for cancer development and growth. In other words, hyperinsulinemia is a compensatory response to control blood glucose levels within the normal range in people who

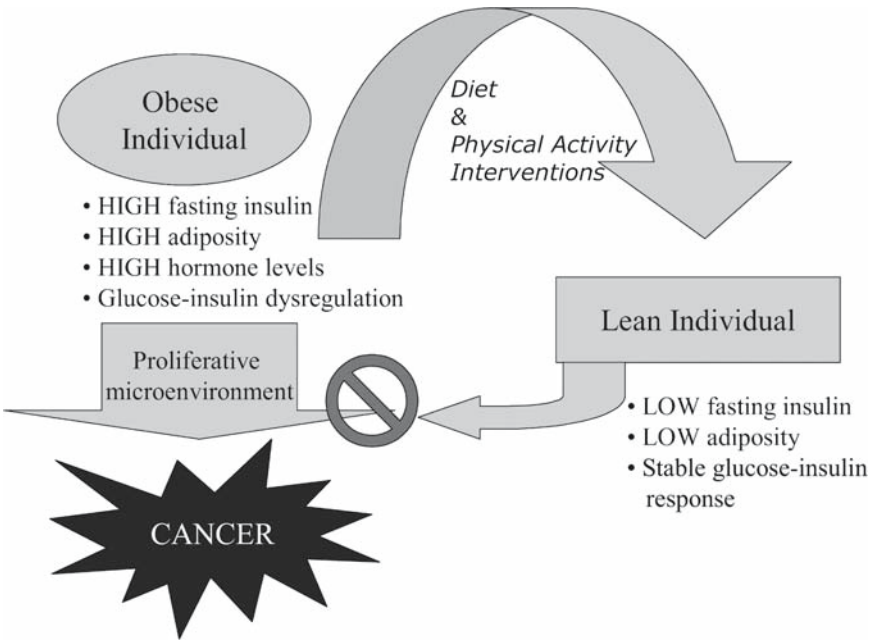


**Fig. 1** Systemic factors modifying cancer risk in obese versus lean individuals

demonstrate a blunted response to insulin. Evidence suggests that hyperglycemia and, in some cases, the corresponding insulinemia, are associated with increased risk of several cancer types including colorectal, breast and prostate cancers. Elevations in triglycerides, C-peptide (a marker of pancreatic insulin secretion) and insulin-like growth factor 1, and reductions in select insulin binding proteins, have also been associated with increased cancer risk in both animal models and human trials, thus further supporting the insulin resistance theory of carcinogenesis (Reneham and Berster 2008; Pollack 2007).

In turn, dietary selections and eating patterns can further augment this cancer-promoting process. It has been shown that diets high in refined sugars and carbohydrates elevate blood glucose levels and insulin response more so than diets high in complex or high fiber foods. Intake of protein- or fat-containing food items along with refined carbohydrates will reduce the maximal height on the insulin response curve as measured in peripheral blood over time, but will not prevent the rise all together. In addition, the normal biological control of blood glucose and insulin response is significantly reduced in the presence of adiposity and with aging. Certain populations, such as the Pima Indians, may have a genetic predisposition toward insulin resistance. Cell culture studies using a wide variety of cancer cell lines exposed to exogenous insulin and/or simple sugars, such as sucrose and high fructose corn syrup, also indicate that carcinogenesis is promoted in such an environment.

Both optimal dietary selections and increased physical activity can modulate both the potential for developing insulin resistance as well as reverse the process in those previously demonstrated to have insulin resistance (Pollack 2007). Weight loss is pivotal to these protective effects. Significant reductions in energy intake (10–20% of energy requirements) should be prescribed for weight loss of one-half to one kilogram weekly until weight is within normal acceptable limits. Concurrent with energy restriction, emphasis should be placed on increasing energy expenditure, starting with a goal expenditure of 1,000 kcal per week and increasing to 2,500–3,000 kcal per week. Weight loss is the single most effective intervention to prevent or reverse insulin resistance. Although the evidence does not currently exist regarding the specific macronutrient composition of the diet to prevent or control insulin resistance, the biologic and pathophysiologic features of the insulin resistance syndrome indicate that a diet restricted in simple sugars, including high fructose corn syrup and refined carbohydrates, is warranted. Efforts to increase high-fiber foods, which are low in sugars, and to consume a protein or fat food source, along with any carbohydrate-rich food, may also be of benefit. It is important to note that there are circumstances when a person of seemingly normal body weight may demonstrate increased adiposity, particularly central adiposity, and thus be prone to insulin resistance syndrome. In these cases, dietary modification as described above should be considered; however, optimizing physical activity levels will be central to reversing the adverse effects of hyperinsulinemia.

*Immune Modulation*. Cancer is thought to be a disease of the immune system. In fact, recent evidence from the Women's health initiative prospectively showed a positive association between elevated white blood cell count (an indicator of inflammation) and several cancers. Including breast, colorectal, endometrial and lung cancers (Margolis et al. 2007). Theoretically, if the immune system is functioning optimally, cancer should not occur. To this end, research has focused both on understanding the role of the immune system in cancer prevention as well as how lifestyle behaviors can modulate immune response. Several nutrients have been shown to play a role in immune function ranging from protein, the building block of immune

cells, to numerous micronutrients, such as vitamins A, C, D, E, and zinc and copper. The role of select nutrients in immune modulation is summarized in Table 3.4. The appropriate level of nutrient intake for promoting optimal immune response has not been clearly established. For some nutrients it appears that there is a window; intake below or above this range may have immunosuppressive effects (e.g. zinc). For other nutrients, levels must be significantly depressed before immune suppression is shown (e.g. vitamin A). What is clear is that among immunosuppressed populations (such as HIV-infected individuals), the risk for cancer is increased. In addition, these same populations generally demonstrate improved immunity in response to nutrient-dense diets or supplementation. However, whether the provision of nutrients to these high-risk individuals also reduces cancer occurrence has yet to be established.

**Table 3.4** Role of nutrients in enhancing immunity

| Nutrient | Food sources | Demonstrated effects on immune response |
|---|---|---|
| **Vitamins** | | |
| Vitamin A | Fortified dairy, yellow-orange vegetables | Improve mucosal integrity, increast T cell function, increase antigen (AG)-specific immunoglobin-G response |
| Vitamin C | Citrus, peppers, broccoli | Increase T cell response, increase phagocytosis, increase epithelial integrity |
| Vitamin E | Seeds, almonds, oils, Raisin Bran | Increase cytokin production, increase B cell function, increase T cell cytotoxicity, increase phagocytosis |
| Vitamin D | Fish oils, fortified dairy products | Increased T cell response |
| **Minerals** | | |
| Copper | Beef liver, cashews, molasses | Increase B cell function, increase T cell response, increase phagocytic function |
| Iron | Fortified cereals, liver, clams | Increase B cells, increase ab production, increase lymphoid tissue |
| Magnesium | | Increase cytotoxic cells, increase cytokine production |
| Zinc | Oysters, wheat germ, dark meat, poultry | Increase B cell function, increase cytokine production, increase cell mediated immunity |
| **Other Nutrients** | | |
| Omega-3 fatty acids | Salmon, cold water fish, flax | Decrease inflammatory response |
| Protein | Lean meat, low-fat diary, egg white | Increase total lyphocyte count/response to Ag, increase epithelial integrity |

In general, it is important to assess the adequacy of the diet in terms of the immune-modulating nutrients. Particularly for individuals at increased genetic risk for cancer, optimizing micronutrient intake is a feasible and potentially health promoting approach to reducing cancer risk. Although the risk may remain, dietary modification to promote intake of a nutrient-dense diet may delay disease onset or reduce disease severity. In fact, a recent study of lifestyle behaviors among women with BRCA1 and BRCA2 genes demonstrated a 10- to 13-year delay in onset of breast cancer among women with a healthy body weight and particularly among those who reported regular physical activity (King et al. 2003).

As discussed earlier, obesity is a contributing factor in the development of several cancers. It has been demonstrated that obese individuals have suppressed immune response. Not only are the numbers of CD4- and CD8-positive T cells reduced, macrophage response is delayed in obese subjects as is natural killer cell cytotoxicity. It therefore appears that one plausible mechanism by which cancer risk is elevated in obese individuals may be related to the resultant immunosuppression, the onset of which is related to an accumulation of adipose tissue.

*Inflammatory Response*. One factor relevant to immune response and cancer risk theoretically identifies cancer as an inflammatory disease. In fact, inflammatory biomarkers have been shown to be elevated in a variety of cancer patients before, during and after cancer therapy (Naugler and Karin 2008). Inflammation results in cellular damage and thus is thought to be a contributory factor in the multi-step pathway to carcinogenesis. Inflammation is also characterized by an accumulation of macrophages that in turn release reactive oxygen species, another contributing factor in cancer development. This biological response may be of particular importance to the obese patient who not only demonstrates reduced macrophage-related response to antigens, but also accumulates macrophages locally in adipose tissue thus elevating the level of localized oxidative damage in these cells.

There are several naturally-occurring inflammatory response modifiers in the human diet. Of particular interest are the omega-3 fatty acids. Fatty acids have demonstrated effects on membrane fluidity and eicosanoid production that in turn alters signal-transduction pathways, membrane-bound receptors and enzyme activity. The end result of these biological effects is an alteration in cytokine release and inflammatory response. Increasing omega-3 fatty acid intake results in a reduction in omega-6 fatty acid and in particular arachidonic acid, a pro-inflammatory compound. Fish oil, an abundant source of omega-3 fatty acids, when supplemented in the diet, results in a significant decrease in the omega-6 to omega-3 fatty acid ratio. Omega-3 fatty acids and foods high in omega-3 fatty acids, including alpha linolenic acid, are considered anti-inflammatory and thus may play a role similar to prescribed cyclooxygenase inhibitors in reducing cancer risk. Recent evidence also suggests that responsiveness to omega-3 fatty acid supplementation may be modulated by genetic background (Fig. 2).

*Oxidative Damage*. Related to the inflammatory response is the role of oxidative DNA damage in cancer development and progression (Bartsch and Nair 2006). All living organisms consuming oxygen experience oxidative damage to tissue on an ongoing basis. In turn, within the human, adaptive mechanisms exist to either reverse the oxidative damage inherent to our biological processes or minimize the effects by clearing byproducts of oxidative damage from the body, thus reducing cancer risk. The role of antioxidants in reducing oxidative damage and promoting repair has been well described in the literature (Ames 1983; Halliwell 2002). Many clinicians fail to recognize that beyond the more well-known antioxidants (e.g. vitamins C and E and selenium) are several naturally-occurring phytochemicals that also have significant antioxidant properties. These include carotenoids found in vegetables and

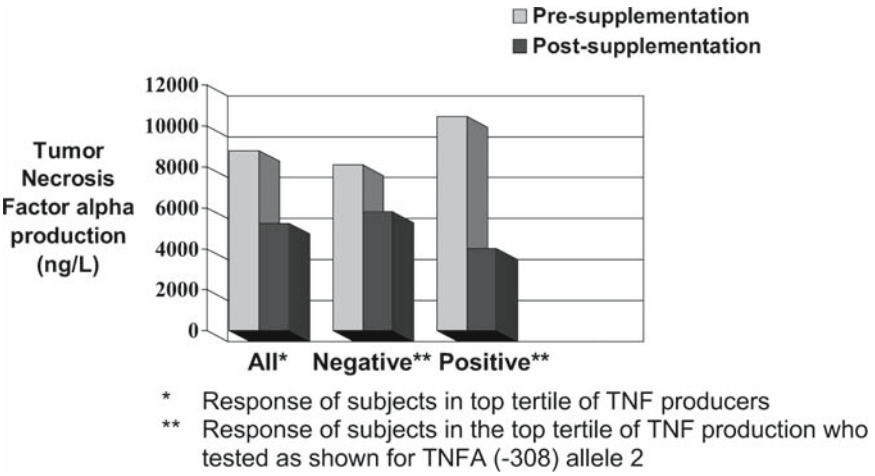


**Fig. 2** Modulation of inflammatory response with omega-3 fatty acid supplementation (Adapted from Grimble et al. 2002)

fruits, polyphenols found in teas, resveratrol found in grapes, limonene from citrus peel, or isoflavones found in soy foods. The cancer preventive role of vegetables and fruits likely stems in part from the high antioxidant content.

Several studies have been published over the past 5–10 years demonstrating the potential for vegetables and fruit to reduce oxidative damage. In a study by Thompson and colleagues (Thompson et al. 1999), a controlled feeding of vegetable (carrot) juice resulted in a significant reduction in oxidative damage biomarkers among healthy individuals. Another study showed a similar response using a high vegetable and fruit diet (Djuric et al. 1991). Similar results have been shown while feeding a variety of foods rich in antioxidant properties, not only to healthy individuals but also to smokers, people diagnosed with cancer and long-term cancer survivors (Djuric et al. 1998; van Zeeland et al. 1999; Collins and Harrington, 2002).

Reduction in oxidative damage through dietary modification seems an appropriate approach to reducing cancer risk. However, there is no direct evidence that such an approach will result in reduced cancer rates. A prudent diet should include a wide variety of vegetables and fruits with attention to a broad range of food colors. The diet should also include additional food selections that promote greater intake of dietary constituents that have demonstrated antioxidant properties such as green tea, citrus peel, onion, garlic, and soy foods.

*Diet–gene Interactions.* Briefly, it is important to mention the role genetics is likely to play in the future of cancer-preventive diets and lifestyle interventions for individuals and sub-groups at risk. A person's responsiveness to dietary intervention is increasingly being identified as being affected by the complexity of an individual's genotype and genetic polymorphisms. It is likely that many of the inconsistencies demonstrated in the epidemiological assessment of relationships between dietary constituents and specific cancer endpoints may be explained by genetic variability. In a given population, the interplay between an individual's genotype and dietary exposures throughout the lifespan may significantly influence whether or not that individual is diagnosed with cancer (Fig. 3). Understanding this relationship, future dietary interventions can be targeted to those at risk and can be adapted for individual likelihood of response.

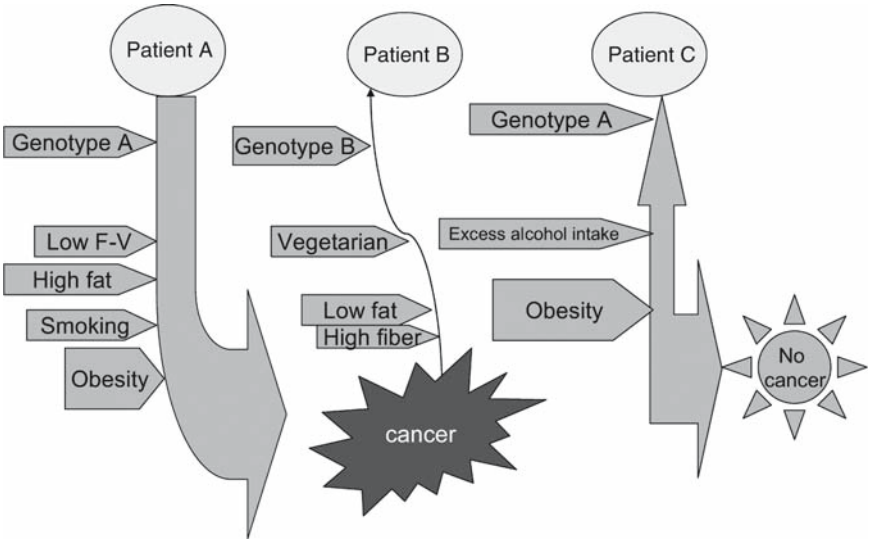


**Fig. 3**

## 3.13 Biological Mechanisms by Which Physical Activity May Reduce Cancer Risk

Whereas the exact biological mechanisms defining the relationship between physical activity and cancer remain unknown, many plausible explanations have been identified. It is likely that multiple mechanisms exist that demonstrate individual variability in cancer risk reduction, based upon genetics, environment, cancer type and stage, and the form of physical activity performed.

*Insulin, Glucose and Insulin-Like Growth Factor*. Elevations in insulin and insulin-like growth factor-1 (IGF-1) have been associated with increased rates of many cancers (LeRoith et al. 1995). IGF-1 is down regulated by increased synthesis of a binding protein (IGFBP-3) which is enhanced by physical activity. Decreased IGF-1 may also lead to increases in sex hormone binding globulin (SHBG), thereby leading to decreases in unbound sex steroids. Finally, a strong relationship exists between physical activity and circulating levels of insulin and insulin sensitivity. This relationship may also be mediated through adiposity.

*Body Composition and Obesity*. Obesity and fat distribution have been associated with increased rates of many cancers. Abdominal fat, specifically visceral or intra-abdominal fat, is the most metabolically active fat store. This relationship is mediated through variations in hormone levels, to include sex steroid hormones, insulin and IGF-1. Physically active individuals are generally neither obese nor demonstrate central distribution of body fat (Westerlind 2003). Visceral fat is preferentially affected by aerobic exercise (Schwartz 1991). Calorie restriction has been demonstrated to have a protective effect (Kritchevsky 1999) and although compensatory mechanisms often exist for calorie expenditure from

exercise, it is plausible that this relationship may exist to a degree in individuals who are physically active.

*Immune Function*. The effects of physical activity on the immune system have often been held as a primary link between physical activity and cancer, although it is believed that the majority of cancers are non-immunogenic (Westerlind 2003). Regular, moderate exercise and physical activity have been noted to affect a number of immune parameters, both numerically and functionally, to include: macrophages, natural killer cells (NK), cytotoxic T lymphocytes, and lymphokine-activated killer cells (LAK) (Newsholme and Parry-Billings 1994). Aging-associated decreases in immune function (immune senescence) have been suggested as a possible explanation for the increased rates of cancer seen with aging. Conversely, regular physical activity has been noted to enhance T-cell function of elderly men and women (Mazzeo 1994). Therefore, overall immune enhancement and slowing of immune senescence may represent the physical activity-immunity relationship.

*Oxidative Damage*. Physical activity and exercise demonstrate varying degrees of oxidative damage and generation of free radicals. Whereas moderate activity causes no to minimal damage in young and/or trained athletes (Margaritis et al. 1997), strenuous exercise may increase rates of oxidative stress (Poulsen et al. 1996). Free radicals may adversely affect DNA and may stimulate mutagenesis and tumor proliferation (Dreher and Junod, 1996). Moderate physical activity and training effects may enhance the body's innate antioxidant system and scavenging of free radicals. Conversely, intense exercise may overwhelm the body's ability to manage oxidative stress, leading to increased oxidative damage.

*Steroid Sex Hormones*. Steroid sex hormones are associated with the development of reproductive cancers in both women and men. The varied effects of exercise on these hormones is believed to be responsible for this protective relationship. Epidemiologic evidence generally supports an inverse relationship between physical activity and the incidence of prostate cancer (Oliveria and Lee 1997), although it has been noted that there is an inverse relationship between upper body mass and prostate cancer, possibly due to higher testosterone levels from increased muscularity (Severson et al. 1988). It has been identified that chronic endurance activities may decrease levels of testosterone, although this effect has not been reported consistently (Lucia et al. 1996; Hackney et al. 1998). A relationship has been identified between sedentary occupations and increased risk of testicular cancer (Coggon et al. 1986), whereas 15 or more hours of vigorous physical activity per week has been noted to decrease the risk of this cancer (1994). Concentrations of SHBG may be increased, thereby leading to depressed levels of free circulating testosterone.

Likewise in women, SHBG levels may demonstrate a similar response to exercise. Additional mechanisms may lead to decreases of both estrogen and progesterone, in premenopausal women, causing increased menstrual irregularities, a shortened luteal phase, and increased anovulatory cycles (Westerlind 2003). Decreased hormone levels have also been identified in post-menopausal women that appear to be unrelated to body fat (Nelson et al. 1988).

It is generally noted that physical activity decreases bowel transit time, possibly mediated by increased vagal tone and increased peristalsis. This may lead to decreased

exposure time of toxins with the bowel mucosa and inhibit promotion of bowel-associated carcinogenesis.

## 3.14 Advancing the Guidelines for Cancer Preventive Lifestyle

Currently, the guidelines provided by the American Cancer Society for primary cancer prevention and recurrence (Kushi et al. 2006) and from the WCRF/AICR report (WCRF/AICR 2007) should serve as the foundation of any recommendations provided to individuals to reduce their cancer risk. Individualization is essential, based upon the person's physical status, prior physical activity and exercise history, and goals and expectations.

The following is an expansion of current dietary and physical activity recommendations based on evolving scientific evidence. While prospective hypothesis-driven intervention trials are not available to support all of these recommendations, the risk-benefit ratio of available evidence suggests these recommendations should be considered in providing lifestyle advice to people seeking to reduce their cancer risk.

- Achieve and maintain a healthy body weight and body composition
  - Be aware of small, incremental changes that over time result in excess adiposity
  - Restrict energy intake
  - Avoid energy-dense foods including sugary beverages, animal fats and fast foods
  - Control food portion sizes
  - Increase energy expenditure
  - Perform both aerobic and weight-bearing activities daily
- Eat more vegetables daily
  - Include a dark green leafy vegetable daily
  - Include a dark orange-yellow vegetable daily
  - Include cruciferous vegetables
  - Include allium vegetables (onion, garlic, leeks)
  - Do not overcook vegetables or saturate in high fat sauces/condiments
- Eat at least three servings of fruit daily
  - Consume fresh fruit over juice
  - Include one serving of citrus daily
  - Include berries, cherries or other dark red-purple fruits daily
- Eat at least 30 g of fiber daily
  - Select both soluble and insoluble fiber sources
  - Select only whole grain breads and cereals
- Reduce intake or avoid processed or refined carbohydrates
  - Select whole grain breads
  - Read labels and avoid foods with greater than 10 g of sugar per serving

- Avoid foods and beverages containing high fructose corn syrup
- Prepare food at home more; reduce meals from fast food restaurants
- Include foods rich in antioxidants
  - Vegetables and fruits
  - Teas
- Avoid processed meat and limit red meat to less than two servings per week
  - Do not over cook meat
  - Select lean cuts
- Include omega-3 rich foods daily
  - Cold-water fish
  - Flax seed
  - Omega-3 oils
- Adopt a physically active lifestyle

*Adults*: Engage in at least moderate aerobic physical activity for at least 30 min on most, preferably all, days of the week. Recognition of the exercise continuum indicating that health and fitness gains are enhanced by greater volumes of activity should serve as a goal to achieve at least 20–60 min of continuous or intermittent (minimum of 10 min bouts accumulated throughout the day) moderate to vigorous aerobic physical activity regularly (Pollock et al. 1998; USDHHS 1996).

*Adults*: Engage in resistance training 2–3 days per week. A minimum of 8–10 exercises involving the major muscle groups should be performed that incorporate a minimum of one set of 8–15 repetitions.

*Youth*: Accumulate at least 60 min, and up to several hours, of age-appropriate moderate-to-vigorous physical activity on most, preferably all, days of the week (NASPE 2004).

All individuals should incorporate more physical activity into their daily lives. Simple measures such as the following can enhance regular activity and improve health:
The American Cancer Society identifies specific issues for survivors of cancer that may preclude their participation in physical activity (Brown et al. 2003).

- Take the stairs rather than the elevator or escalator
- "Actively commute" by walking or bicycling where and when appropriate
- Schedule active family outings and vacations
- Engage in moderate housework and yardwork where and when available
- Take 10-min minimum walk breaks at work
- Wear a pedometer to gauge daily activity level
- Get together regularly with friends and/or family members for hikes or walks
- Walk the dog
- Take the first available parking space and walk to the store entrance
- Carry bags of groceries in from the car one at a time
- Jump rope or run in place during television viewing
- Sit on a balance ball while working at your computer

- Individuals with anemia should refrain from activity, other than that of daily living, until anemia improves.
- Individuals with compromised immune systems should avoid public gyms and other public venues; survivors post-bone marrow transplantation should avoid these spaces for one year following transplant.
- Persons experiencing significant fatigue should listen to their bodies and do as much as they feel able to do and are encouraged to do 10 min of stretching daily.
- Individuals should avoid chlorinated pools if undergoing radiation therapy.
- Persons with indwelling catheters should avoid water or other microbial exposures that may result in infections. They should also avoid resistance training that may cause dislodgement of the catheter.
- Persons who are experiencing significant peripheral neuropathy that may impede their ability to perform exercises and activities that make use of the affected limbs may consider using a recumbent bicycle or similar exercise equipment in controlled settings rather than performing activities outdoors.

As previously mentioned, it is important for clinicians to individualize physical activity and exercise recommendations as determined by the unique situation of the patient. An emphasis should be placed upon getting all individuals to limit their sedentary behavior and increase their activity as tolerated.

It is also imperative that the above lifestyle practices begin early in life and be sustained throughout life. Parents must become strong role models of healthy behavior for their children and communities must provide environments that are supportive of such behaviors.

## 3.15 Tools for Research and Clinical Practice

Researchers and clinicians can benefit from having the appropriate tools necessary to integrate diet and nutrition as well as physical activity and body composition assessment and behavior change instruments available. The remainder of this chapter focuses on key issues in measurement, assessment and implementation of behavior change in this context. Some will have greater application to the researcher, while others will be most appropriately applied in clinical practice.

## 3.16 Measuring Diet

Dietary measurement is among the most challenging issues facing researchers and clinicians alike. Current dietary measurement tools rely heavily on self report. It has been demonstrated that people have difficulty accurately recalling dietary intake and due to social desirability may have significant discomfort in reporting intake even when accurately recalled. This is particularly true for overweight persons, and has been primarily described among women.

There are three major approaches to measuring diet reported in the scientific literature. These include: the Food Frequency Questionnaire (FFQ), where individuals recall intake of a specific list of foods (80–250 items), frequency of intake, and approximate serving sizes; dietary recalls, where individuals report their intake in terms of food items, amounts and preparation methods for one or more 24-h periods; and the dietary record, where individuals record all food consumed, amounts, brands and preparation methods for a pre-defined period of time. Each approach has both strengths and weaknesses. Correlations between these approaches generally range between 30 and 60% supporting the concern for significant reporting error when it comes to dietary measurement. However, until more accurate approaches are developed, these approaches remain the basis for much of the dietary measurement reported in the literature. Several cancer centers across the US (e.g. University of Arizona, Fred Hutchinson Cancer Research Center, University of North Carolina, and University of Minnesota) have shared services resources to assist clinicians and researchers in selecting the most appropriate instruments and approaches for use.

Generally, to best assess intake, researchers recommend that more than one approach be used and when possible, biological markers of intake also be measured. Presented in Table 3.5 are several biomarkers of dietary intake that can be employed in an effort to validate self-reported intake.

In addition, investigators have developed several focused food frequency questionnaires to more accurately capture intake of select foods that have been recognized for their chemopreventive properties. These include the Citrus Intake Questionnaire, the Arizona Tea Questionnaire, the Soy Foods Questionnaire and most recently the Cruciferous Vegetable Questionnaire (Several of these instruments are available through the Dietary and Behavioral Measurements Service at the University of Arizona at www.azdiet-behavior.azcc.arizona.edu). These focused instruments allow for more complete and valid assessment of

**Table 3.5** Biological markers (assays) used to validate self-reported dietary intake

| Dietary constituents of interest | Biomarker/functional biomarker |
|---|---|
| Fiber | Fecal hemicellulose;fecal weight, short-chain fatty acid |
| Folate | Plasma folate; red blood cell folate; plasma homocysteine |
| Vitamin B12 | Plasma B12 |
| Vitamin C | Plasma vitamin C; urine deoxypridinoline |
| Vitamin E | Plasma tocopheros; LDL oxidation |
| Calcium | Bone density; serum osteocalcin |
| Selenium | Plasma or whol blood selenium; toenail selenium; plasma GSH peroxidase activity |
| Carotenoids | Plasma levels (alpha- and beta-caroten; lutein; lycopene |
| Cruciferous Vegetables/isothiocyanates | Urinary dithiocarbamates |
| Citrus/limonene | Urinary perillylic acid |
| Tea polyphenols | Urinary polyphenols |

**Table 3.6** Dietary measurement websites

| Organization | Website address | Components |
|---|---|---|
| Arizona Cancer Center Diet and Behavioral Measurement Service | http://www.azdiet-behavior.azcc.arizona.edu | Food Frequency Questionnaires including FFQ's for Tea, Citrus and Cruciferous Vegetables |
| Block, Gladys- Investigator | http://www.nutritionquest.com/fat-screener.html | Fat Screener |
| National Cancer Institute | http://riskfactor.cancer.gov/diet/screener/fruitveg/ | Fruit and Vegetable Screener |
| US Department of Agriculture | http://www.usda.gov/cnpp/healthyeating.html | Healthy Eating Index |

intake of the designated foods, thus supporting more reliable assessment of the association between these foods and cancer risk reduction.

Other clinical and research tools are available online. These include the National Cancer Institute FFQ, Block Fat Screener, and the NCI Fruit and Vegetable Screener. The US Department of Agriculture also has an online Healthy Eating Index; individuals can enter daily dietary intake and can receive an immediate assessment of specific macro and micronutrients, a graphic comparison of how their intake compares to the Food Guide Pyramid, and a summary of individual intake versus national dietary goals. The Healthy Eating Index also affords the individual an opportunity to track progress over time as new dietary behaviors are adapted. A summary of diet evaluation resources is listed in Table 3.6.

## 3.17 Assessment of Physical Activity and Energy Expenditure

Researchers should be familiar with the various methods of assessing physical activity and energy expenditure. The former term is broadly defined as any bodily movement that is produced by the contraction of skeletal muscle and that substantially increases energy expenditure. The latter specifically addresses the energetic cost associated with a specific physical activity and is dependent upon the numerous processes and personal characteristics (e.g., age, body mass, fitness level) associated with that activity (Montoye et al. 1996).

*Physical Activity Questionnaires*. Physical activity questionnaires are simple and inexpensive methods of obtaining data from participants. As they are dependent upon individual self report, they are subject to recall and social bias. They are nonetheless useful and are often used in conjunction with more objective measures.

Global short surveys (1–5 questions) that focus upon general aspects of physical activity provide limited information and lack specificity, but provide an overview of physical activity patterns that may provide background information for more focused studies. Recall, or longer surveys (10–20 questions), are often used to obtain baseline data for comparison with post-intervention data. These instruments provide more detailed information about specific aspects of physical activity (e.g. frequency, amount) over a defined period of time (e.g. days, weeks, months). Quantitative history tools generally have more questions (greater than 20) and provide very detailed information about physical activity patterns over longer periods of time (e.g. past year, period in lifetime, entire lifetime). These instruments address the volume of activity and allow for the determination of dose-response effects on outcome measures of interest (e.g. kcal per week).

*Physical Activity Logs, Records and Recalls*. Physical activity records provide an ongoing account of an individual's physical activity during a defined period of time in an attempt to capture all forms of activity. Physical activity logs are often used to determine adherence to specific protocols. They call for identifying specific aspects of physical activity over determined intervals (e.g. every 15 min). Physical activity recall is usually performed by telephone or personal interviews and attempt to catalogue an individual's physical activity patterns over a defined period of time (e.g. day, week).

*Indirect Measures of Energy Expenditure*. There are a wide variety of tools and methods that can be applied in either laboratory or field situations. They provide reliable and valid measures of free living situations but vary in their logistical burden of performance. Doubly labeled water (DLW) provides an accurate assessment of energy expenditure based upon the volume of carbon dioxide ($VCO_2$) produced and oxygen uptake volume ($VO_2$). Stable isotopes of water are consumed, and then fractional excretion is calculated through urinary measures. Energy expenditure is calculated from determination of $VCO_2$ and $VO_2$. Although an accurate measure, it cannot provide specific information about the characteristics of physical activity that have contributed to the energy expenditure, such as intensity, frequency or duration. $VO_2$ estimates energy intake based upon equations that provide a relationship between oxygen utilization by tissues and caloric utilization by activity (one liter $O_2$ approximately equals 5 kcal). These estimates are commonly determined by a treadmill or bicycle ergometer and are often used to provide an individual with an exercise prescription that defines the volume and intensity of activity to be performed (e.g. 60–90% of maximum heart rate, or 50–85% of $VO_{2max}$).

Heart rate is a commonly used measure that provides an indirect estimate of workload or energy expenditure. It is based upon a linear relationship that may exist between heart rate and $VO_2$, but as it is significantly affected by a number of parameters, its accuracy is less than other measures. It nonetheless is easily measured and is a useful measure in interventions that provide an exercise prescription to participants.

Motion detectors are mechanical instruments that are worn to quantify a measure of physical activity. These instruments are based upon the premise that motion is related to energy expenditure. Accelerometers have commonly been utilized and provide researchers with an accurate assessment of activity intensity and volume over a determined time interval. Limitations, such as the inability to measure activities that may not cause trunk movement (e.g. resistance training), prevent them from accurately providing a measure of the wearer's total energy expenditure.

Pedometers are simple devices that have become increasingly popular for physical activity assessment. They measure the number of steps that are taken while worn, but cannot differentiate characteristics of step-based activities, nor can they measure other forms of activity accurately (e.g., bicycling). Pedometers are inexpensive and have been associated with an increase in motivation toward behavior change, thus making them popular for clinical practice and research. In general, research studies are more likely to rely on accelerometers to measure physical activity because they have a higher correlation with actual energy expenditure [r = 0.34–0.49 (Freedson and Miller 2000) versus r = 0.66–0.96 (Sherman et al. 1998)].

## 3.18 Measurement of Body Composition

*Anthropometric Measurements*. Anthropometry has been used to predict body composition in laboratory and field situations. Some examples of anthropometric variables relevant to body composition are weight, trunk depth, stature, arm span, knee height, breadth of biacromial, bi-iliac, knee, ankle, elbow and wrist, circumference of waist, hip, thigh, calf, arm and wrist, and skinfold thickness at the subscapular, midaxillary, suprailia, triceps and biceps. These variables can be used to predict percent body fat, body density, fat-free mass, total body muscle mass, and total body bone mineral content. A number of body composition predictive models with anthropometric variables have been developed and cross-calibrated. Because anthropometric procedures are non-invasive and the instruments used for anthropometric measurements are portable and relatively inexpensive, anthropometry tends to be used for large population-based studies. However, in comparison to other laboratory techniques for body composition measurements, anthropometric measurements may be less accurate.

*Bioelectric Impedance Analysis (BIA)*. BIA is often a substitute or supplement to conventional anthropometry in field research or epidemiologic studies on body composition. Impedance is the frequency-dependent opposition of a conductor to the flow of an alternating electric current and reflects both resistance and reactance. The use of BIA to estimate body composition is based on the assumption that different body tissue components have different conductive and dielectric properties at different frequencies of current. The conductive and dielectric properties can be measured through impedance. All BIA devices are composed of three essential parts: alternating electrical current sources; cables and electrodes to introduce the current into the body and to send the voltage drop due to impedance; and a system for measuring impedance. BIA is portable and easy to use. Predictive equations of impedance can be developed and calibrated for estimating total and regional body composition, including fat and fat-free mass. However, the general applicability of BIA is often limited by the availability of appropriately calibrated and cross-validated predictive equations in different populations who have different hydration status and thickness of subcutaneous fat. These factors may significantly affect the precision of BIA assessments. Although limited evidence suggests a significant correlation between changes in total body water assessed from

BIA and deuterium dilution among some cancer patients (Simons et al. 1999), BIA does not provide reliable measurements of body composition for cancer patients with ascites (Sarhill et al. 2000). Until more research has been conducted, caution should be taken when applying general BIA predictive equations for body composition estimations among cancer patients.

*Dual-energy X-ray Absorptiometry (DXA)*. A new generation of bone densitometry not only measures bone density, but also measures soft tissue mass. Body soft tissue measurements by DXA are derived from the assumed constant attenuation of pure fat and of bone-free lean tissue. The advantages of DXA in measuring body composition include its qualities as a non-invasive, highly precise and accurate instrument, and that it emits very low radiation (less than a standard x-ray). DXA scans may provide total body and regional measurements of bone mineral content, bone mineral density, soft tissue mass, lean soft tissue mass, and fat tissue mass as well as percent fat tissue mass. Hydration levels and body thickness have very small effects on the precision of DXA-derived body composition assessments. Hence, DXA can be used in both general and patient populations. With new developments in DXA hardware and software, the total body scan time has been significantly shortened, and body composition analyses can be conducted for any defined regions by the investigator. However, the high cost of DXA units and the need of special radiologic training for DXA operators limit the application of DXA for body composition measurements in standard research and clinical practice. Nevertheless, given the growing numbers of DXA instruments, and especially DXA-mobile units, the utility of DXA in field and epidemiologic and clinical studies of body composition will increase. Body composition measurement will likely have an increasing role in the supportive care of the patient, specifically to evaluate nutritional status and treatment effects on health.

*Other Techniques*. Hydrodensitometry, hydrometry, whole-body counting, neutron activation analysis, ultrasound, and imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), are other techniques for body composition assessments. The hydrodensitometry method, which measures percent body fat, requires that the subject completely submerge in water and assumes consistent densities of the constituents of the body from person to person. The hydrometry method measures total body water, as well as intra-cellular and extracellular water, based on the distribution of isotopic tracers in different water compartments. Whole-body counting and neutron activation analysis can be used to measure skeletal muscle mass and other body composition through assessing natural potassium concentration, or assessing selectively activated atoms in the body. Ultrasound has been used to measure regional body composition for over 30 years. However, the precisions of ultrasound-derived body composition measurements are less satisfactory in comparison with DXA measurements.

Most previous studies have used body mass index or weight and waist-to-hip ratio as proxies for obesity and fat distribution. Measurement errors due to improper anatomical placement of the measuring tape and other technician operation errors, particularly with repeated measures, may limit the ability to detect an association of obesity or fat distribution with cancer.

## 3.19 Measurement of Bone Health

The World Health Organization (WHO) criteria for the diagnosis of osteoporosis are based on DXA measurements of BMD of the hip and spine for post-menopausal women. The criteria are T-scores that indicate the number of standard deviations below or above the average peak bone mass in young adults of the same sex (Table 3.7).

The National Osteoporosis Foundation (NOF) Guidelines suggest that all women age 65 years or more, post-menopausal women with fractures, and younger post-menopausal women with one or more risk factors have a bone density test. The NOF suggests initiating therapy for women who have a T-score below −2.0 by central DXA and no additional risk factors, a T-score below −1.5 by central DXA with one or more additional risk factors, or for women with prior vertebral or hip fractures (Wei et al. 2003). According to the International Society for Clinical Densitometry (ISCD), the WHO criteria should neither be applied to healthy premenopausal women nor applied in its entirety to men. The ISCD recommends using Z-scores, rather than T-scores, for osteoporosis diagnosis in premenopausal women. Z-scores indicate the number of standard deviations below or above the average population bone mass, determined by age and gender. The diagnosis of osteoporosis in premenopausal women should not be made on the basis of densitometric criteria alone. Osteoporosis may be diagnosed for premenopausal women with low BMD and secondary causes (e.g. glucocorticoid therapy, hypogonadism, hyperparathyroidism) or risk factors for fracture. The criteria for osteoporosis diagnosis among men may differ by age: in men age 65 and older, T-scores should be used and osteoporosis diagnosed if the T-score is at or below −2.5; between age 50 and 65, T-scores may be used and osteoporosis diagnosed if both the T-score is at or below −2.5 and other risk factors for fracture are identified; and for men under age 50, diagnosis should not be made on densitometric criteria alone. Men at any age with secondary causes of low BMD (e.g., glucocorticoid therapy, hypogonadism, hyperparathyroidism) may be diagnosed clinically with osteoporosis supported by findings of low BMD. For young adults and children (age 20 or under) the value of BMD in predicting fractures is not clearly determined. Z-scores may be used and the diagnosis should not be made on the basis of densitometric criteria alone. “Low bone density for chronological age” may be stated if Z-score is below −2.0 (2004).

There are many different techniques, including DXA, ultrasound devices, and quantitative CT (Maricic and Chen 2000) for central or peripheral BMD measurements. Both the NOF and the ISCD recommend using central DXA scans for osteoporosis diagnosis so as

| T-score | Classification |
|---|---|
| −1 or higher | Normal |
| −1 to −2.5 | Osteopena (or low bone density) |
| −2.5 or lower | Osteoporosis |
| −2.5 or lower + facture | Severe osteoporosis |

**Table 3.7** World Health Organization (WHO) criteria for diagnosis of bone status

**Table 3.8** Biochemical markers of bone health

| Markers of Bone Formation | Osteoblast-dervied enzymes |
|---|---|
| | • Total alkaline phosphatase<br>• Bone-specific alkaline phosphatase, Bone matrix formation product<br>• Osteocalcin<br>• Type 1 collage propeptides |
| **Markers of bone resorption** | **Osteoclast-derived enzymes** |
| | • Acid phosphatase<br>• Tartrate-resistant acid phosphatase, bone matrix degradation products<br>• Collagen cross-links (pyridinoline, deoxypryidinoline, N-telopeptide, C-telopeptide)<br>• Hydroxyproline |

to be in agreement with the WHO criteria. Forearm (33% radius) BMD should be measured for osteoporosis diagnosis under the following circumstances: hip and or spine cannot be measured or interpreted; hyperparathyroidism; and very obese patients (over the weight limit for DXA tables) (ICSD 2004).

*Biomarkers for Bone Metabolism*. Biochemical bone markers reflect bone metabolism or bone turnover. There are two types of bone biomarkers – markers of bone formation and markers of bone absorption (Table 3.8).

When used in conjunction with BMD measurements, the measurement of bone biomarkers may assist clinical decision making regarding the initiation and maintenance of therapy. However, the clinical use of bone biomarkers is complicated by multiple sources of variability related to biological factors and to the assay itself. Some examples of biological factors that may influence these markers include age, sex, ethnicity, physical activity, diet, drug therapy, medical conditions, and time of specimen collection. Assay variability can arise from variations in specimen processing, assay precision and accuracy, standardization, cross-reaction with other organ markers, non-Gaussian distribution, and interlaboratory variation. Therefore, bone biomarkers are poorly accepted by clinicians (Stepan, 2003) and should be used only as secondary or experimental measures of bone density. The value of using biomarkers in aiding treatment decision for osteoporosis or monitoring treatment effects for osteoporosis among cancer patients have yet to be investigated.

## 3.20 Conclusion

Success usually comes when behavior change is made using small, positive and incremental steps that are frequently reinforced. Food and other lifestyle choices are influenced by several factors including perceived risk, cost, convenience, taste (e.g. food), social support,

self-efficacy, and environment. Table 3.9 provides examples of behavior strategies or 'prescriptions' that can be employed to promote behavior change and, as a result, achieve the desired health outcome.

The role of diet, physical activity and body composition continues to be an area of active research both in terms of primary prevention and in terms of reducing morbidity and mortality among those previously treated for cancer. Evidence to date supports efforts to improve dietary selections toward a more plant-based, low fat, complex carbohydrate-rich diet along with daily, regular and varied physical activity. Maintenance of a healthy body weight throughout life is strongly recommended to reduce cancer risk. Measuring diet, physical activity and body composition is both plausible and challenging. Practitioners should develop strategies to routinely evaluate the diet, physical activity and body composition of each patient to promote early and effective behavior change to reduce cancer risk.

**Table 3.9** Desired health outcome and possible behavioral strategies

| Health or behavioral outcome sought | Behavioral strategy |
|---|---|
| Weight loss | • Cut all portions in half |
| | • Use a salad plated to serve food |
| | • Restrict fast food restaurants to once/week |
| | • Record intake |
| | • Substitute calcium chews for dessert |
| Increased fruit and vegetable intake | • Select new fruit or vegetable from the produce department each week |
| | • Purchase a 5-a-day cookbook and try 3 new recipes each week |
| | • Keep a fruit bowl readily available at work and home |
| | • Select at least 5 different colors of produce at the maket each week |
| | • Visit the Farmer's market weekly-bring a friend |
| | • Add fresh fruit to cereal |
| | • Have a fruit smoothie for breakfast |
| | • Put blended vegetables into your pasta sauce |
| | • Make salads a meal |
| Increased fiber intake | • Select and eat cereal with at least 6 grams of fiber per serving |
| | • Try oatmeal again |
| | • Purchase the heaviest bread on the shelves |
| | • Eat five to nine servings of vegetables and fruits daily |

(continued)

**Table 3.9** (continued)

| Health or behavioral outcome sought | Behavioral strategy |
|---|---|
| | • Add seeds to cereal, salads, etc |
| | • Snack on air-popped popcorn |
| Normal glucose-insulin levels | • Avoid any food with great than 10 grams sugar/ serving |
| | • Switch from soda to fresh lemondade or tea with no added sugar |
| | • Consume protein-carbohydrate combined meals |
| | • Replace white bread with whole grain; replace white potato with sweet potato |
| Daily Physical Activity | • Wake up 30 min early and walk |
| | • Find a friend to walk with |
| | • Jump rope during television commercials |
| | • Garden |
| | • Ride a bike to work or on errands |
| | • Try a new sport |
| | • Train for a charity walk/run |
| | • Keep an active log-reward yourself when goals are met |

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# Innate and Adaptive Immune Responses to Cancer

# 4

**K.T. Hastings**

Together, innate and adaptive immune responses are capable of recognizing and destroying cancer. Natural killer cells and gamma-delta T cells are capable of specifically recognizing and killing tumor cells. Tumor antigens have been identified from many cancers, and cancer patients generate CD4+ and CD8+ T lymphocytes and B lymphocytes specific for these antigens. Tumor-specific lymphocytes are found infiltrating tumors and in the peripheral blood of cancer patients. The presence of brisk tumor infiltrating lymphocytes in primary melanoma portends a survival advantage compared to tumors where tumor infiltrating lymphocytes are absent. In some instances, the immune system can cause spontaneous regression of tumors. For example, partial regression is a common finding in primary melanoma lesions, and there are rare reports of complete regression of metastatic melanoma. The importance of the immune system in preventing cancer is reflected in the increased frequency of malignancies in immunosuppressed and immunodeficient patients. Under the selective pressure of the immune response, tumors evolve to escape immune-mediated destruction.

## 4.1 Importance of the Immune System in Cancer Prevention

The concept of cancer immunosurveillance was first proposed by Burnet in 1957 (Burnet 1957) and has been refined into a three step cancer immunoediting process of elimination, equilibrium and escape (Dunn et al. 2002). Elimination refers to cancer immunosurveillance and the ability of the innate and adaptive immune responses to suppress tumor formation. In mouse models, the absence of the cytokine interferon (IFN)-γ or IFN-γ signaling

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(IFN-γ receptor knockout or signal transducer and activator of transcription 1 (STAT1) transcription factor knockout) results in enhanced formation of transplanted tumors and chemically-induced and spontaneous tumors (Dighe et al. 1994; Kaplan et al. 1998). IFN-γ is produced by natural killer (NK) cells as part of the innate arm and by T lymphocytes as part of the adaptive arm of the immune response and will be discussed in detail later. In addition, perforin-deficient mice are more prone to develop chemically-induced and spontaneous tumors than wild-type mice (van den Broek et al. 1996; Smyth et al. 2000; Street et al. 2001; Street et al. 2002). Perforin is a component of the cytolytic granules of both NK cells and CD8 + cytotoxic T lymphocytes (CTLs) and, thus, is a mediator of target cell killing for both the innate and adaptive immune responses. Recombination activating gene (RAG) 1 and 2 are essential for rearrangement of the B and T cell receptors, and deficiency of either enzyme results in the complete absence of B and T cells. RAG-deficient mice have an increased frequency of chemically-induced and spontaneous tumors (Shankaran et al. 2001). Mice with combined deficiencies in the lymphocyte pool (RAG knockout or perforin knockout) in addition to IFN-γ have a modest increase in tumor formation (Shankaran et al. 2001; Street et al. 2001). In addition, mice with deletions of NK cells and T cells expressing the γδ T cell receptor (TCR) have increased susceptibility to chemically-induced tumors (Girardi et al. 2001; Smyth et al. 2001).

There is corresponding evidence for the role of the immune response in elimination of tumors in humans. Immunodeficient or immunosuppressed patients have an increased incidence of cancer. Many of these cancers have a viral etiology such as Epstein-Barr virus (EBV)-induced lymphomas, human herpesvirus 8 (HHV8)-induced Kaposi sarcoma and human papillomavirus (HPV)-induced cervical, anogenital and skin cancers. However, the incidence of cancers without an apparent viral etiology are also increased. A two to tenfold increase in the incidence of melanoma has been observed in transplant recipients (Sheil 1986; Penn 1996) as well as an increased incidence of colon, lung, pancreatic, endocrine, bladder and kidney cancer (Birkeland et al. 1995; Pham et al. 1995). The presence of lymphocytes within a tumor correlates with increased patient survival. For example in primary cutaneous melanoma there are three categories of tumor infiltrating lymphocytes (TILs): brisk, non-brisk and absent. Figure 1 demonstrates the histopathology of a primary cutaneous melanoma with brisk TILs defined as lymphocytes forming a continuous band beneath the tumor or diffusely distributed thoughout its substance. The patients with brisk TILs have a one and a half to three times longer survival advantage than the patients with absent TILs. Similar positive correlations between TILs and patient survival are seen in breast, bladder, colon, prostate, ovarian, rectal and neuroblastoma cancers.

Equilibrium refers to the immune response controlling the expansion of fully transformed tumor cells. Recent evidence using a mouse model of chemical carcinogenesis demonstrates that the adaptive immune response (neutralizing antibodies against CD4, CD8 and IFN-γ), but not NK cells are responsible for maintaining equilibrium (Koebel et al. 2007). Escape refers to the tumor cell variants that expand in an uncontrolled fashion and grow into clinically apparent tumors. Using mouse models of chemically-induced tumors, spontaneously arising tumors from wild-type mice form progressively growing tumors in either RAG knockout or wild-type mice (Dunn et al. 2002). In contrast, tumors that grow in RAG knockout mice are rejected by naïve, wild-type mice demonstrating increased immunogenicity of tumors developed in the absence of adaptive immunity (Dunn et al. 2002).

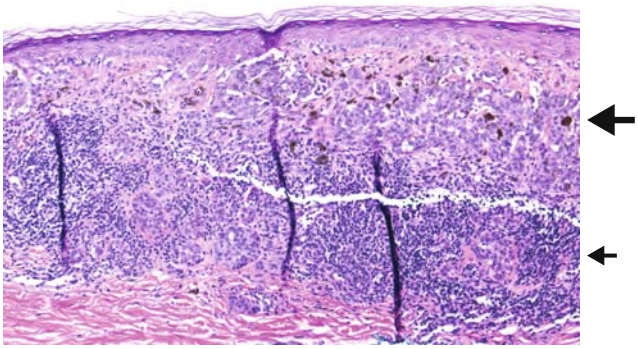

**Fig. 1** Tumor infiltrating lymphocytes in a malignant melanoma. Punch biopsy specimen of a cutaneous malignant melanoma demonstrating epidermal and dermal nests of melanoma cells (*large arrow*) and the presence of brisk tumor infiltrating lymphocytes forming a band at the base of the tumor (*small arrow*). (Hematoxylin-eosin stain; ×100 magnification) Photo courtesy of Christine J. Ko, M.D., Yale University

Chemically-induced sarcomas maintained in equilibrium in wild-type mice grow when transplanted into RAG knockout mice (Koebel et al. 2007). Taken together, this evidence demonstrates a role for the immune response in controlling tumor growth and in selecting tumor cells variants with decreased immunogenecity for escape.

## 4.2 Innate Immune Responses to Cancer

The innate immune response represents the first line of defense against cancer and infection. Cells of the innate immune response recognize patterns as opposed to the antigen-specific immune responses of adaptive immunity. Innate immunity has the ability to recognize tumors and contributes to immunosurveillance and destruction of tumors. In the first step of the elimination process, tumors begin to require increased blood supply, and invasive growth induces inflammatory signals that lead to the recruitment of cells of the innate immune response including NK cells, γδ T cells, macrophages and dendritic cells (DCs). NK cells and γδ T cells secrete IFN-γ and are able to kill tumor cells. In the next phase of the elimination process, IFN-γ induces a limited amount of tumor death through anti-proliferative and pro-apoptotic mechanisms and induces the production of chemokines and adhesion molecule expression resulting in enhanced leukocyte infiltration and tumor killing. DCs which have ingested tumor cell debris travel to the draining lymph node to stimulate naïve, tumor-specific CD4+ and CD8+ T lymphocytes. In the final stage of elimination, activated, tumor-specific T cells home to the tumor site, secrete IFN-γ and destroy tumor cells.

4

### 4.2.1 Natural Killer Cells

Natural killer (NK) cells destroy malignantly transformed cells, but usually do not damage healthy cells (Lanier 2005; Gasser and Raulet 2006). NK cells distinguish dangerous targets from normal cells using a combination of inhibitory and activating receptors. The inhibitory receptors share the presence of immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic tails. Upon engagement of the receptor, the ITIM is phosphorylated and recruits phosphatases such as SHP-1, SHP-2 or SHIP which counteract the effect of kinases in the signaling pathway initiated by activating receptors. Activating receptors lack ITIMs in their cytoplasmic tail and are generally associated with DAP12. The balance of signals from inhibitory and activating receptors determines NK cell activation and target cell killing. Similar to CTLs, NK cells kill target cells using granzymes, perforin, CD95L (FasL) and tumor necrosis factor (TNF)-related apotosis inducing ligand (TRAIL). NK cells also express the low affinity Fc receptor for IgG (CD16) which enables NK cells to kill target cells coated with IgG via antibody-dependent cell-mediated cytotoxicity.

Human NK cell major histocompatibility complex (MHC) class I-specific inhibitory receptors include members of at least three families: killer cell immunoglobulin-like receptor (KIR), leukocyte immunoglobulin-like receptor (LILR) (also known as LIR, ILT and CD85) and CD94/NKG2 families (Lanier 2005; Gasser and Raulet 2006). Yet each of these families has some members which can serve as activating receptors. The polymorphic KIR family recognizes different alleles of MHC class I molecules HLA-A, -B and -C. KIRs bind both the MHC class I molecule and its bound peptide. Some KIRs have short cytoplasmic tails without ITIMs. These activating KIRs either do not bind class I or bind with much lower affinity. The LILR family recognizes many MHC class I alleles. LILRB1 is an inhibitory receptor with four ITIMs in its cytoplasmic tail and is variably expressed on NK cells and a subset of T cells and highly expressed on B cells and monocytes. LILRB1 binds with low affinity to a conserved region of the $\alpha 3$ domain of essentially all MHC class I alleles (HLA-A,-B,-C,-E,-F and -G). The CD94/natural killer group 2 (NKG2) family of receptors consists of a disulfide-linked heterodimer of C-type lectin NKG2 with CD94. The most well characterized receptors are the CD94/NKG2A inhibitory receptor and the CD94/NKG2C activating receptor. NKG2A has an ITIM in its cytoplasmic domain. In contrast, NKG2C/CD94 acts as an activating receptor and associates with the DAP12 adapter protein for stable cell surface expression and signaling. CD94/NKG2 receptors are expressed on most NK cells and $\gamma\delta$ T cells and a subset of CD8+ CTLs. Both CD94/NKG2A and CD94/NKG2C bind HLA-E, a non-classical MHC class I molecule. Since stable surface expression of HLA-E requires peptide binding and the most abundant peptides bound to HLA-E are the signal peptides from HLA-A, -B, -C or -G, the CD94/NKG2 receptors monitor the presence of classical (HLA-A,-B,-C) and non-classical (HLA-E,-G) MHC class I molecules. MHC class I molecules are constitutively expressed on essentially all nucleated cells. Thus, inhibitory receptors grant NK cells the capacity to attack cells that have lost or downregulated MHC class I expression. Tumor cells frequently have lowered or absent MHC class I expression possibly due to selective pressure to escape lysis by CD8+ CTLs.

NKG2D and NKp46, NKp44, NKp30 are activating receptors that recognize self ligands that are poorly expressed on normal cells and are upregulated on cancer and infected cells (Lanier 2005; Gasser and Raulet 2006). The NKG2D receptor is encoded by a single, non-polymorphic gene. The name is a misleading because the *NKG2D* gene has very little homology to the *NKGA, NKGC, NKGE*, and *NKGF* genes, and the NKG2D protein does not form dimers with CD94. NKG2D is expressed as a disulfide-linked homodimer on the surface of all NK cells, all CD8 + T cells and most γδ T cells. Stimulation of NK cells through NKG2D triggers cell-mediated cytotoxicity and in some cases cytokine secretion. NKG2D binds molecules with structural homology to MHC class I: MHC class I chain related A (MICA), MICB and UL16 binding proteins (ULBP1, also called Raet1), ULBP2, ULBP3 and ULBP4. MIC and ULBP proteins are expressed in response to activation of the DNA damage response and are found on tumor cell lines, precancerous cells, cancer cells and cells infected by some viruses. However, tumors have ways to evade NKG2D-mediated detection. Although tumors frequently express NKG2D ligands, some tumors secrete these ligands. Secreted ligands can serve as decoys to subvert NK cell and T cell immune responses. For example, soluble MICA and ULBP proteins have been detected in the sera of cancer patients, and these individuals have significantly decreased levels of NKG2D expression and impaired activation of NK and T cells. In addition, transforming growth factor (TGF)-β, which is frequently produced by tumors, downregulates expression of NKG2D on lymphocytes (see below).

### 4.2.2 Gamma-Delta T Cells

The majority of T cells express a TCR composed of an α and β chain. A subset of T cells, 2–5% of peripheral blood T cells, express a TCR composed of a γ and δ chain (Kabelitz et al. 2007; Morita et al. 2007). These γδ T cells have similar effector functions to T cells expressing αβ TCRs, such as cytokine secretion (tumor necrosis factor (TNF)-α and IFN-γ) and cytotoxic activity. However, these cells recognize a distinct subset of ligands and may play a role in tumor immunosurveillance and destruction.

γδ T cells bearing the variable region gene Vδ1 can exhibit anti-tumor activity through recognition of MICA, MICB and ULBPs via the NKG2D receptor. There is also evidence that the Vδ1 TCR can directly bind MICA (Wu et al. 2002; Zhao et al. 2006). MICA and MICB are not usually expressed on normal tissue. The intestinal epithelium is an exception, presumably due to constant microbe exposure. Expression of MICA and MICB corresponds with increased frequencies of Vδ1 T cells at this site. Similarly, MICA and MICB expression has been identified in lung, breast, renal cell, ovarian, prostate and colon carcinoma and corresponds to an increased frequency of Vδ1 T cells relative to all γδ T cells (Groh et al. 1999). Vδ1 T cell clones, including those derived from tumors, recognize MICA and MICB on both autologous and allogeneic tumor cells. Vδ1 T cells are increased in chronic lymphocytic leukemia of B cell type (B-CLL) patients with low risk stage compared to high risk stage and healthy donors (Poggi et al. 2004). Vδ1 cells produce TNF-α or IFN-γ in response to autologous B-CLL cells, but not normal lymphocytes. Incidentally,

patients with high numbers of Vδ1 T cells and detectable or inducible ULBP have a better prognosis. Vδ1 T cells have also been shown to have anti-melanoma activity (Lozupone et al. 2004). In addition to anti-tumor activity, recent work in breast cancers has identified a large number of tumor infiltrating Vδ1 T cells with suppressive activity mediated through soluble factors other than TGF-β or interleukin (IL)-10 (Peng et al. 2007).

γδ T cells bearing the variable region gene Vδ2 recognize non-peptidic phosphorylated isoprenoid pathway metabolites referred to as phosphoantigens(Bonneville and Scotet 2006; Morita et al. 2007). The population of Vγ2Vδ2 (also called Vγ9Vδ2) T cells makes up the largest proportion of peripheral blood γδ T cells. The most potent Vγ2Vδ2 antigens, such as hydroxy-methyl-butyl-pyrophosphate (HMBPP), are produced through the 1-deoxy-D-xylulose-5-phosphate pathway found in microorganisms (Jomaa et al. 1999; Begley et al. 2004). In contrast, eukaryotic cells use the mevalonate pathway for isoprenoid biosynthesis and produce isopentenyl pryophosphate (IPP) which activates Vγ2Vδ2 T cells, but only at supra-physiologic concentrations. Unlike peptide antigens recognized by γδ T cells, recognition of phosphoantigens does not require antigen uptake and intracellular antigen processing. In addition, phosphoantigen activation of Vγ2Vδ2 T cells requires cell-cell contact, and the phosphoantigen does not bind directly to the γδ TCR (Lang et al. 1995; Morita et al. 1995). These characteristics suggest the presence of an antigen presenting molecule; however, the known antigen presenting molecules are not required, and an antigen presenting molecule has not been identified (Morita et al. 1995). Tumor cell lines and peripheral blood mononuclear cells from different donors can present phosphoantigens to allogeneic Vγ2Vδ2 T cells (Morita et al. 1995), suggesting that if present, the antigen presenting molecule is non-polymorphic. The Vγ2Vδ2 TCR has also been reported to recognize a mitochondrial F1-adenosine triphosphatase (ATPase) related structure on the surface of a B cell lymphoma line aided by the presence of apolipoprotein A-I in the culture medium (Scotet et al. 2005). Vγ2Vδ2 T cells recognize phoshoantigens via the TCR, but their activity can be modulated via activating receptors such as NKG2D and NKp44 and inhibitory receptors including CD94/NKG2A and KIRs. Therefore, Vγ2Vδ2 T cells, like NK cells, can recognize tumor cells that have upregulated MICA/B and ULBPs or have downregulated MHC class I. In addition, Vγ2Vδ2 T cells are reported to present peptide antigens in the context MHC class II for the stimulation of CD4+ αβ T cells (Brandes et al. 2005).

Vγ2Vδ2 T cells are likely to be important in the anti-tumor immune response. Vγ2Vδ2 T cells are increased in patients with hematopoietic and solid tumors (Bonneville and Scotet 2006). Some patients with lymphoid malignancies have dramatic expansions of γδ T cells in the peripheral blood (McClanahan et al. 1999). In vitro human Vγ2Vδ2 T cells show broad tumor cytotoxicity including bladder cancer (Kato et al. 2001), breast cancer (Guo et al. 2005), colon carcinoma (Corvaisier et al. 2005), B cell lymphoma (Fisch et al. 1997; Sicard et al. 2001), melanoma (Kabelitz et al. 2004), myeloma (Kunzmann et al. 2000; Mariani, Muraro et al. 2005; von Lilienfeld-Toal et al. 2006), nasopharyngeal carcinoma (Zheng et al. 2001), neuroblastoma (Otto et al. 2005), pancreatic adenocarcinoma (Kabelitz et al. 2004), prostate cancer (Liu et al. 2005), renal cell carcinoma (Kobayashi et al. 2001; Viey et al. 2005) and small cell lung cancer (Sato et al. 2005b), and are not limited by MHC restriction like γδ T cells. Human Vγ2Vδ2 T cells transferred into severe combined immunodeficiency (SCID) mice have anti-tumor activity against B cell lymphoma

(Malkovska et al. 1992), nasopharyngeal carcinomas (Zheng et al. 2001), pancreatic adenocarcinoma (Kabelitz et al. 2004), melanoma (Kabelitz et al. 2004; Lozupone et al. 2004), and neuroblastoma (Otto et al. 2005). γδ T cells have the ability to lyse a wide variety of tumors; however, much of this activity is due to recognition of ligands by NK-activating receptors such as NKG2D (Das et al. 2001). Vγ2Vδ2 T cells can recognize some malignant B cells, such as a B cell lymphoma line and plasmacytoma line, directly via the TCR (Fisch et al. 1990; Selin et al. 1992; Bukowski et al. 1995). Of interest, some tumors over-express hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase. Endogenous or induced over-expression of HMG-CoA reductase or treatment with farnesylpyrophosphate synthase inhibitors leads to accumulation of isoprenoid pathway metabolites such as IPP which can be detected by Vγ2Vδ2 T cells (Gober et al. 2003). Both pathways likely contribute to T cell activation and tumor cell lysis, as shown by Vγ2Vδ2 T cell recognition of colon carcinoma that is dependent on both the TCR and NKG2D (Corvaisier et al. 2005). The ability of γδ T cells to recognize a broad range of tumors without MHC restriction and without reactivity to non-transformed cells makes them candidates for the prevention and treatment of cancer.

### 4.2.3 Phagocytes

Dendritic cells (DCs) reside in the tissues and play a key role in initiating and controlling the magnitude and quality of the adaptive immune response (Ueno et al. 2007). Immature DCs act as senitels for potentially dangerous signals from cancer cells or microbes and have strong phagocytic antigen capturing abilities. Upon receiving maturation stimuli, immature DCs lose adhesion molecule expression, undergo cytoskeleton reorganization and migrate to the draining lymph node. In the maturation process, they lose their endocytic and phagocytic receptors and, in turn, become adept at processing previously captured antigens. Mature DCs are professional antigen presenting cells and have increased MHC class II and costimulatory molecule expression on their cell surface. The innate immune response detects microbes using pattern recognition receptors that are germline encoded to recognize a limited number of microbial patterns. These receptors include Toll-like receptors (TLRs), cell surface C-type lectin receptors and intracytoplasmic nucleotide oliomerization domain (NOD)-like receptors. Microbes directly activate DCs through their pattern recognition receptors. There is evidence the DCs can also be activated by products of dying cells, the tissue microenvironment and innate immune cells. Thus, DCs have a critical function in the activation of the adaptive immune response.

DCs can play a role in immune evasion (Ueno et al. 2007). DCs are important in generating peripheral tolerance. Immature DCs are believed to continuously present self antigens sampled from the environment. Engagement of the TCR on an autoreactive T cell in the absence of costimulation results in anergy or deletion of the autoreactive cell. However, tumor antigens that are presented by immature DCs, that have not been activated, will result in anergy or deletion of tumor-specific T cells. In addition, tumors have a number of mechanisms to block DC differentiation and maturation. STAT3 is constitutively

activated in a diverse group of hematologic and epithelial cancers inhibiting tumor production of pro-inflammatory cytokines and promoting release of soluble factors that inhibit DC function. Cytokines in the tumor microenvironment, such as IL-10, interfere with DC maturation and yield tolerogenic DCs that induce tumor-specific anergy. Growth factors in the tumor microenvironment such as vascular endothelial growth factor (VEGF) also inhibit DC maturation. Tumor antigens such as MUC-1 evade efficient MHC class II-restricted processing in DCs and block DC secretion of IL-12 skewing the CD4+ helper T (Th) cell response to the Th2 subset (see below).

Tumor associated macrophages are derived from peripheral blood monocytes and can comprise up to 50% of the tumor mass (Knowles and Harris 2007). Classically activated M1 macrophages, in response to IFN-γ and microbes, have strong antigen processing and presenting capability, cytotoxic activity and high IL-12 secretion. Macrophages are capable of killing tumor cells in the same ways that they kill microbes including the release of lysosomal enzymes, nitric oxide (NO) and reactive oxygen species (ROS). Activated macrophages also secrete TNF, which as the name implies, has anti-tumor activities (discussed below). Increased numbers of tumor associated macrophages correlate with enhanced tumor cell apoptosis and improved disease-free survival in gastric and esophageal cancer.

Alternatively, activated M2 macrophages promote tumor growth and metastasis. They promote angiogenesis, matrix remodeling and suppression of effective adaptive immunity. Furthermore, they lack tumor cell lytic ability and have poor antigen processing and presenting capability. M2 macrophages secrete matrix metalloproteases (MMPs), some of which are induced by the hypoxic tumor enviroment, that digest the tumor extracellular matrix and facilitate endothelial cells migration. The M2 phenotype is promoted by secretion of prostaglandin $E_2$, TGF-β (discussed below), IL-4, IL-6 and IL-10 within the tumor microenvironment. Overactivation of STAT3 can also contribute to the macrophage's immunosuppressive phenotype. Activated monocytes from the peripheral blood and macrophages from ascites of ovarian cancer patients have defects in antibody-dependent cell-mediated cytotoxicity and phagocytosis. In many cancers, especially breast cancer, a high number of tumor-associated macrophages correlates with poor prognosis (Knowles and Harris 2007).

### 4.2.4 Cytokines

Cytokines are polypeptides that are produced in response to microbes and other antigens, such as tumor antigens, and regulate inflammatory and immune reactions. Cytokine secretion is a brief, self-limited event, and cytokines exert their function by binding cytokine receptors on target cells and altering gene expression. The majority of cytokines act locally in an autocrine or paracrine fashion. However, some cytokines may be produced in large amounts, enter the bloodstream and have systemic or endocrine effects, such as TNF in gram-negative bacterial sepsis. Cytokines may be divided into those that are produced by cells of the innate immune response, such as DCs and macrophages, and those that are produced by cells of the adaptive immune response, namely T lymphocytes. TNF, type I

IFNs and IL-12 are key cytokines that mediate and control the innate immune response. Key cytokines that regulate the adaptive immune response include IL-2, IFN-γ and TGF-β and will be discussed in the following section on the adaptive immune response.

*Tumor necrosis factor*. TNF, also called TNF-α, is primarily produced by macrophages that have been activated by TLR engagement (Mocellin and Nitti 2008). TNF enhances leukocyte homing to sites of inflammation by inducing vascular endothelial cell expression of adhesion molecules that are responsible for leukocyte homing, stimulating chemokine secretion by endothelial cells and macrophages that enhance the affinity of leukocyte integrins for their ligands and promote leukocyte homing and chemotaxis, and decreasing endothelial cell intercellular adhesion. TNF increases endothelial cell synthesis of NO and matrix metalloproteinases (MMPs) and increases surface thrombogenecity of endothelial cells. TNF also induces production of other cytokines (such as IL-1 and IL-6) by macrophages and activates macrophages and neutrophils to induce production of NO and ROS.

TNF can mediate selective destruction of tumor cells through two mechanisms (Mocellin and Nitti 2008). TNF is capable of direct cytotoxicity. TNF binding to TNF receptor-1 (TNFR1) leads to the association of adaptor protein TNF receptor-associated death domain containing protein (TRADD) which ultimately leads to apoptosis through caspase activation. TNF may also disrupt tumor vasculature and is responsible for hemorrhagic necrosis of tumors. In animal models, the absence of TNF diminishes tumor rejection, and humans receiving TNF inhibitors as treatment for auto-immune disease have a three to five fold increased risk of developing lymphoma.

In contrast, TNF can promote cancer development and metastasis (Mocellin and Nitti 2008). Association of TNFR1 with other adaptor proteins such as TNF receptor associated factors (TRAFs) stimulates degradation of the inhibitor of NF-κB resulting in activation of NF-κB and promotion of cell survival. Chronic inflammation with persistent, low-dose TNF results in an increase in positive cell cycle regulators (Ras, c-myc) and a decrease in cyclin-dependent kinase inhibitors. This pathway has been shown to be important in the progression of Barrett esophagus to mucosal dysplasia to esophageal adenocarcinoma. Release of genotoxic substances, such as NO and ROS that are induced by TNF, into the environment can promote DNA damage. Chronic low doses of TNF promote angiogenesis required for tumor growth and production of MMPs which contribute to tumor invasion. In animal models, exogenous TNF or tumor-induced TNF produced by tumor infiltrating macrophages promotes metastasis.

The pleotropic nature of TNF can be explained by the fact that TNF can stimulate multiple intracellular pathways that mediate opposing effects (Mocellin and Nitti 2008). In addition, the effect of TNF on tumors depends on the tumor microenvironment: the degree of vascularization, the presence of an MMP family member which cleaves TNF, the amount of NO produced by tumor endothelial cells. In general, chronic low doses of TNF favor angiogenesis and tumor development and progression, and single, high doses cause destruction of newly formed blood vessels and tumor regression. Clinical trials with TNF alone or as an adjuvant have not demonstrated a survival benefit.

*Type I interferons*. A principal function of type I IFNs, including IFN-α and IFN-β is to mediate the early innate immune response to viral infections through inducing the expression of proteins that inhibit viral replication (Stark et al. 1998; Bracci et al. 2007).

In addition, type I IFNs stimulate a number of functions important for the recognition and destruction of cancer and infected cells. The major source of IFN-α is plasmacytoid DCs, and the most potent stimuli for type I IFN synthesis are viral nucleic acids which bind to cytoplasmic sensors, caspase activation and recruitment domain (CARD)-containing proteins called retinoic acid inducible gene-1 (RIG-1) or melanoma differentiation-associated gene 5 (MDA5). Macrophages are also a source of type I IFNs, and IFN production is stimulated through the interaction of CD40L on activated T cells with CD40 on macrophages. TLR ligation can also stimulate type I IFN production. IFN-β is produced by many cell types, including fibroblasts.

Type I IFNs promote innate and adaptive immune responses (Stark et al. 1998; Bracci et al. 2007). Type I IFNs serve as an important signal for differentiation and maturation of DCs for antigen presentation to CD4+ and CD8+ T cells. They also promote the development of, activation of, and NO production in macrophages. Type I IFNs upregulate IFN-γ production by DCs and T cells favoring the differentiation of CD4+ T cells into the Th1 subset (see below). Type I IFNs promote the generation and activity of CD8+ T cells as well as enhance the lytic activity of activated CD8+ T cells for targets, including tumor cells. Type I IFNs promote the survival and proliferation of CD8+ and CD4+ T cells though expression of anti-apoptotic genes and induction of IL-15 by antigen presenting cells. Type I IFNs also increase the cytotoxicity of NK cells.

Type I IFNs also exert effects on tumor cells and infected cells to promote their destruction (Stark et al. 1998; Bracci et al. 2007). All IFNs increase the expression of MHC class I molecules, and thus increase recognition of and killing by CD8+ CTLs. This effect is important not only for destruction of virally infected cells, but also for destruction of tumor cells. Type I IFNs have an anti-proliferative effect inhibiting cell growth by targeting components of the cell cycle and important in the suppression of cancer and infection. A distinct type I IFN-mediated pathway controls apoptosis exerting either anti- or pro-apoptotic effects depending on the state of cell differentiation.

IFN-α augments the immune response and can inhibit tumor cell proliferation, downregulate oncogene expression, induce tumor suppression genes and increase MHC class I expression to improve immune recognition (Stark et al. 1998; Bracci et al. 2007). Tumor cells transfected with IFN-α are rejected more effectively. These anti-tumor effects of IFN-α are taken advantage of by using it as a cancer treatment. IFN-α is used clinically in the treatment of cutaneous T cell lymphoma, multiple myeloma, malignant melanoma, renal cell carcinoma and HHV8 associated Kaposi sarcoma.

*Interleukin-12*. IL-12 is a key inducer of the innate immune response and the adaptive immune response for defense against cancer and intracellular microbes (Trinchieri 2003). IL-12 is a heterodimer composed of p35 and p40 subunits and is produced primarily by activated DCs and macrophages. It induces the differentiation of naïve CD4+ T cells into Th1 cells (see below) and maintains Th1 responses which promote cell-mediated immunity. IL-12 stimulates production of IFN-γ from NK cells and T cells. It enhances the cytotoxic functions of activated NKs cells and CD8+ CTLs.

The anti-tumor effect of IL-12 depends on CD4+ and CD8+ T cells as well as a contribution from suppression of angiogenesis (Del Vecchio et al. 2007). Tumor cells transfected with IL-12 are rejected by CD8+ CTLs and accompanied by macrophage infiltration, vascular damage and necrosis. Treatment with IL-12 induces IFN-γ production within the

tumor microenvironment, and IL-12's anti-tumor properties are dependent on its ability to induce IFN-γ expression and intact signaling through IFN-γ receptors (see below). Systemic delivery of IL-12 has remarkable efficacy in not only tumor prevention, but in the treatment of well-established tumors in animal models. In light of IL-12's anti-tumor effect in pre-clinical studies, IL-12 has been investigated as a monotherapy or as an adjuvant. Clinical trials of systemic IL-12 for the treatment of cutaneous T cell lymphoma, acquired immunodeficiency syndrome (AIDS)-related Kaposi sarcoma and non-Hodgkin lymphoma have an objective response rate of 21–56%. The efficacy is minimal in the remainder of the clinical trials using IL-12 for the treatment of other advanced solid tumors.

## 4.3 Adaptive Immune Response

### 4.3.1 Recognition of Tumor Antigens

A large number of tumor antigens recognized by T and B cells have been identified. Antibodies have tremendous diversity, specificity and affinity for the antigens that they recognize. Antibodies can recognize proteins/peptides, nucleic acids, polysaccharides, lipids and small chemicals. Antibodies can recognize conformational or linear epitopes, and antibodies can bind soluble or cell-associated antigens. The antigen specificity of antibodies generated by cancer patients has been determined using serologic analysis of recombinant complementary DNA expression (SEREX). In SEREX, the patient's tumor RNA is used to generate a complementary DNA (cDNA) expression library, and the patient's serum immunoglobulins are used to screen the library and determine the gene sequence and predicted protein product recognized.

The majority of T cells recognize linear peptides presented by cell-surface major histocompatibility complex (MHC) class I or class II molecules. Epitopes recognized by T cells have been identified by cloning T cells derived from cancer patients' peripheral blood, tumor-draining lymph node or TILs. A cDNA library is prepared from the tumor cells, pools of DNA from the library are transfected into syngeneic cells, and the T cell clones are tested for reactivity against the transfected cells. Smaller pools of DNA can be transfected until a single gene is identified. Similarily, synthetic peptide fragments can be used to isolate the T cell epitope. A comprehensive database of known T cell epitopes of tumor antigens is available at http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm. In addition, a subset of T cells recognize lipid antigens presenting via CD1 or phosphate-containing antigens.

Nearly every nucleated cell including tumor cells expresses MHC class I and can present antigens derived from the cytoplasmic compartment for recognition and lysis by CD8+ CTLs (Cresswell et al. 2005). The proteasome degrades cytosolic proteins, and the resulting peptides can be transported into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP). Assembly of MHC class I heavy chain with $\beta_2$-microglobulin ($\beta_2$M) and a cytosolically generated peptide occurs in the ER and

is assisted by several chaperones. The MHC class I-$\beta_2$M-peptide complex is transported to the cell surface where it can be recognized by the TCR on CD8+ T lymphocytes. A tumor cell uses this classical MHC class I pathway to present its antigens to CD8+ CTLs. However, naive CD8+ T cells must first be stimulated by a professional antigen presenting cell and receive signals from costimulatory molecules and cytokines to become activated. Cross-presentation or cross-priming refers to the ability of professional antigen presenting cells such as DCs to present material derived from the extracellular space, such as tumor cell debris, on MHC class I for the stimulation of naive CD8+ CTLs. Phagocytosed proteins reach the cytoplasmic compartment, are degraded by the proteosome, translocated into the ER via TAP and loaded onto MHC class I. Tumors can evade immune recognition through disrupting MHC class I-restricted antigen processing through loss of class I itself or components in the class I pathway (Algarra et al. 2004). For example, some melanomas lose cell surface expression of MHC class I through defective expression of $\beta_2$M, which is required for stable assembly of class I, or defective expression of TAP (Wang et al. 1996).

MHC class II is expressed primarily by professional antigen presenting cells, can be upregulated by IFN-$\gamma$ on other cells types, and can be aberrantly expressed on some tumors such as melanoma, lung cancer, breast cancer, and osteosarcoma. The MHC class II pathway produces cell surface MHC class II-peptide complexes for the activation of CD4+ T lymphocytes (Hiltbold and Roche 2002). MHC class II $\alpha\beta$ heterodimers are formed in the ER and associate with invariant chain (Ii). The C-terminal portion of Ii (CLIP) protects the class II peptide binding groove from acquiring peptide in the ER like class I, and the N-terminal cytoplasmic domain of Ii targets the class II-Ii complex to endosomes and lysosomes. Exogenous proteins enter the endocytic pathway via pinocytosis, phagocytosis and receptor-mediated endocytosis. Endogenous proteins normally located in the endocytic compartment are also presented on MHC class II. In the late endosomes and lysosomes, gamma-interferon-inducible lysosomal thiol reductase (GILT) reduces disulfide bonds of proteins in the endocytic compartment exposing additional epitopes for class II binding, and proteases further breakdown protein antigens for presentation by class II. The class II-related molecule HLA-DM facilitates the exchange of CLIP for an antigenic peptide, and the MHC class II-peptide complexes are directed to the cell surface.

### 4.3.2 Tumor Antigens

Tumor antigens can be classified based upon their molecular structure and expression.

*Tissue-specific differentiation antigens*. Tumor cells express lineage-specific differentiation antigens that are also expressed in the cells of origin (Abeloff et al. 2004). For example, melanocyte differentiation antigens including tyrosinase, tyrosinase-related protein-1 and -2, gp100 and melan-A/MART-1 are expressed by both benign and malignant melanocytes. These melanoma antigens are melanosomal proteins involved in melanin biosynthesis. Since these antigens are self-antigens also expressed in normal tissues, they tend to elicit tolerance. Overexpression in melanoma cells may contribute to the ability of the immune system to mount a response to these self-antigens. Melanoma patients generate CD4+ T lymphocytes, CD8+ T lymphocytes and B cells specific for these antigens.

Since these antigens are not essential for tumor growth or survival, immune responses to these tumor antigens exert selective pressure that results in the survival and outgrowth of tumor cells that have lost expression of tissue-specific differentiation antigens. This mechanism of immune evasion is observed in melanomas through the loss of melanocyte differentiation antigen expression and escape from melanocyte differentiation antigen-specific CTLs (Trefzer et al. 2006).

Prostate-specific antigen (PSA) is an example of a tissue-specific differentiation antigen that is useful in cancer diagnosis. PSA is a serine protease expressed at a high level in luminal epithelial cells of the prostate and is absent or present at low levels in other tissues (Abeloff et al. 2004). Expression is regulated by androgens. Serum levels increase with age and are higher in African American men compared to Caucasian men. Serum levels of PSA are elevated in prostate cancer and can also be seen in benign prostatic hypertrophy. PSA levels, along with digital rectal examination, detect prostate cancer at very early stages; PSA levels are also helpful in management after diagnosis. Two MHC class I-restricted peptides from PSA have been shown to stimulate CTL activity (Correale et al. 1997).

Other examples of tissue-specific differentiation antigens are CD10 and CD20, which are expressed on B cell precursors, mature B cells and B cell-derived lymphomas (Abeloff et al. 2004). These cell surface antigens help identify the cell of origin and are useful targets in immunotherapy. Rituximab is an anti-CD20 monoclonal antibody approved for the treatment of non-Hodgkin lymphoma and other B cell malignancies. Although tissue-specific differentiation antigens are usually not tumor-specific, tumor-specific examples are the idiotypic determinants of the B cell receptor (immunoglobulin) and T cell receptor (TCR). Each B and T cell receptor has a unique antigen recognition site which is specific to that B or T cell clone.

*Tumors antigens resulting from mutations*. Many tumors express mutated gene products that are required for malignant transformation or maintenance of the malignant phenotype. These antigens represent excellent targets for the immune response, because the mutated gene products are tumor-specific, and therefore not subjected to immune tolerance. In addition, since many of these mutations are required for continued growth of the cancer, the cancer cells are not able to evade immune-mediated destruction through loss of these antigens.

Mutations in oncogenes essential for tumorigenesis are important tumor-specific targets of the immune response. The *Ras* proto-oncogene is a guanosine triphosphate (GTP)-binding protein with a low level of GTPase activity that is active when bound to GTP and inactive when bound to guanosine diphosphate (GDP) (Schubbert et al. 2007). Ras proteins are negatively regulated by GTPase activating proteins (GAPs) which markedly increase the intrinsic GTPase activity. Ras-GTP regulates a signal transduction network resulting in increased transcription due to alternations in nuclear transcription factors. The most well-characterized Ras effector pathway is Raf-mitogen-activated and extracellular-signal regulated kinase (MEK)-extracellular signal-regulated kinase (ERK) cascade leading to formation of the activator protein 1 (AP1) transcription factor. Activating *Ras* mutations impair the intrinsic GTPase activity and response to GAPs, such that Ras accumulates in the active form. Activating *Ras* mutations are found in approximately 30% of human cancers. The *Ras* family contains *KRAS*, *NRAS* and *HRAS. KRAS* mutations are common in pancreatic, colorectal, endometrial, biliary tract, lung and cervical cancers.

A mutated K-Ras peptide recognized by CD8 + CTLs has been identified in pancreatic cancer (Gjertsen et al. 1997). *KRAS* and *NRAS* mutations are seen in myeloid malignancies. Mutated *NRAS* and *HRAS* are prevalent in melanoma and bladder cancer. A mutated N-Ras peptide is recognized by CD8 + CTLs in melanoma (Linard et al. 2002).

Chronic myelogenous leukemia (CML) is characterized by a balanced translocation between Abelson (*ABL*) proto-oncogene on chromosone 9 and breakpoint cluster region (*BCR*) on chromosome 22 (Abeloff et al. 2004). The 210 kD chimeric protein product (p210) exhibits constitutive tyrosinse kinase activity compared to the tightly regulated tyrosine kinase activity of the normal *ABL* product. The junctional region of the Bcr-Abl chimeric protein represents a novel, CML-specific antigen that is recognized by both CD4 + and CD8 + T lymphocytes (Bosch et al. 1996; Yotnda et al. 1998; Makita et al. 2002).

Mutations in tumor suppressor genes also represent important tumor-specific antigens. The p53 tumor suppressor gene is mutated in approximately 60% of cancers, and mutations of upstream or downstream signaling molecules are found in the remainder (Bourdon 2007). The p53 gene encodes nine different isoforms which function as transcription factors that stop cell cycle progression for DNA repair or promote apoptosis of damaged cells. It is interesting to note that 60% of p53 mutations do not affect transcriptional activity, 15% lead to completely inactive transactivation, and 25% have differential transcriptional activity. CD4+ and CD8+ T cell epitopes have been identified from p53, but they do not involve mutations (Fujita et al. 1998; Azuma et al. 2003).

Tumor suppressor *CDKN2A* encodes two separate gene products, p16 and p14 alternate reading frame (ARF) (Otto et al. 2005), which are both involved in negative regulation of cell cycle progression (Abeloff et al. 2004). The p16 protein is a competitive inhibitor of cyclin-dependent kinase 4 (CDK4). CDK4 interacts with cyclin D and phosphorylates the retinoblastoma (Rb) protein which leads to S phase progression. *CDK2NA* mutations leading to loss of p16 function increase the probability that mutated DNA is not repaired prior to cell division. The p14ARF protein binds to MDM2 resulting in stabilization of p53 and $G_1$ arrest. Loss of p14ARF function leads to increased destruction of p53 and enhanced growth of mutated cells. p16 is mutated in 30–50% of familial melanoma and 25–40% of sporadic melanomas, and p16 mutations have been identified in other solid tumors and hematologic malignancies (Ruas and Peters 1998). A novel peptide epitope caused by a two base pair deletion in *CDK2NA* exon 2 is recognized by CD8+ T cells from a melanoma patient (Huang et al. 2004). Less frequent mutations in melanoma are observed in *CDK4*, and a mutated CDK4 peptide was identified as a tumor-specific antigen in melanoma recognized by CD8+ T cells (Wolfel et al. 1995). This mutation was found in 2 out of 28 melanomas tested.

The *wnt-1* proto-oncogene activates β-catenin signaling by reducing the rate of β-catenin degradation, whereas *adenomatous polyposis coli* (*APC*) tumor suppressor gene (mutated in colon cancer) enhances β-catenin degradation. A mutated β-catenin epitope that enhances stabilization is recognized by CD8+ T cells and present in tumors isolated from multiple melanoma patients (Rubinfeld et al. 1997). Mutations in genes that are not involved in tumorigenesis have also been identified. Multiple mutated proteins have been identified in melanomas that are recognized by CD8+ CTLs including *ARTC1*, *FN1*, *GPNMB*, *MART-2*, *MUM-1,2* and *3*, *neo-PAP*, *myosin*, *PRDX5*, *PTPRK*, *RBAF600*, *SIRT2*, and *SNRPD1* (Coulie et al. 1995; Chiari et al. 1999; Zorn and Hercend 1999; Baurain et al.

2000; Kawakami et al. 2001; Topalian et al. 2002; Wang et al. 2002; Novellino et al. 2003; Lennerz et al. 2005; Sensi et al. 2005; Wang et al. 2005).

*Abnormally expressed cellular proteins*. Cancer/testis antigens, which are normally expressed in gametes and trophoblasts, are found in many types of cancer, but not in normal somatic tissues (Scanlan et al. 2002; Nicholaou et al. 2006). Approximately 20 cancer/testis antigens or antigen families have been identified. In general, these antigens are not required for the malignant phenotype and are not mutated. Instead, they are distinguished by abnormal expression. They are expressed on a wide variety of cancers including bladder, breast, hepatocellular carcinoma, melanoma, multiple myeloma, neuroblastoma, non-small cell lung, ovarian, prostate and thyroid. The X chromosome contains a disproportionately high number of the cancer/testis antigens. In fact, up to 10% of the genes on the X chromosome are cancer/testis antigens. Expression of these antigens appears to be due to demethylation of the promoter regions and/or histone acetylation. The function of the majority of these antigens is not known. The first cancer/testis antigen was identified as an antigen recognized by patient-derived melanoma-specific CD8+ CTLs and termed melanoma antigen-1 or MAGE-1. MAGE-1 was subsequently renamed as MAGE-A1 with the discovery of the MAGE-A and MAGE-B families each with at least 15 members. Cancer/testis antigen NY-ESO-1 was identified by SEREX from the serum of a patient with esophageal squamous cell carcinoma. LAGE-1, the second member of the NY-ESO-1 family with approximately 85% amino acid sequence homology, was cloned by mRNA expression profiling. MAGE and NY-ESO-1 family members generate specific antibody responses in cancer patients, and a large number of CD8+ and CD4+ T cell epitopes from MAGE family members, NY-ESO-1 and LAGE-1 have been identified.

The proto-oncogene *ERBB2 (Her-2/neu)* encodes a transmembrane receptor tyrosine kinase (Abeloff et al. 2004). *ERBB2* gene amplification or protein overexpression is found in 20–30% of breast cancers and is the basis for treatment with trastuzumab, a humanized monoclonal antibody that blocks ERBB2 signaling. Multiple Her-2/neu MHC class I binding epitopes recognized by CD8 + T cells have been identified (Fisk et al. 1995; Scardino et al. 2002).

*Antigens of oncogenic viruses*. Products of oncogenic viruses function as tumor-specific antigens and elicit T cell response that help to eliminate tumor cells (Abeloff et al. 2004). EBV-associated lymphomas, HPV-associated squamous cell carcinomas of the skin, HPV-associated cervical and anogenital cancers and HHV8-associated Kaposi sarcoma arise more frequently in immunosuppressed individuals such as allograft recipients on immunosuppressive therapy and patients with AIDS. HPV E6 and E7 proteins are expressed in infected cells, and they inactivate tumor suppressors p53 and pRB, respectively, contributing to carcinogenesis. The development of lymphoma in mice transgenic for the *EBNA-1* gene of EBV, requirement for EBNA-1 for the malignant phenotype of a Burkitt lymphoma cell line, and association of *EBNA-1* mutations with Burkitt lymphoma suggests that the EBNA-1 may contribute to lymphomagenesis.

*Oncofetal antigens*. Oncofetal antigens are expressed in fetal development and cancer cells; however, they are present at lower levels in normal adult tissues and in non-neoplastic conditions. The two most characterized oncofetal antigens are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). CEA (CD66) is a highly glycosylated transmembrane protein that is a member of the immunoglobulin superfamily (Abeloff et al. 2004). CEA functions as a homophilic intercellular adhesion molecule. CEA is normally expressed at

high levels in the gastrointestinal tract, pancreas and liver during the first 2–6 months of fetal development. CEA is expressed at low levels in normal adult colonic mucosa and the lactating breast. CEA expression and serum levels can be elevated in many epithelial-derived carcinomas including colon, pancreas, stomach and breast. Although serum levels of CEA are not useful for the diagnosis of primary tumors, when serum levels of CEA are elevated initially, they are useful for monitoring tumor progression during treatment. CEA levels can be modestly elevated (usually less than 10 ng $ml^{-1}$) in smokers and patients with benign gastrointestinal and hepatic disorders (Loewenstein and Zamcheck 1978).

AFP is a circulating glycoprotein that is normally synthesized and secreted by the yolk sac and liver during fetal development (Abeloff et al. 2004). AFP is the major serum protein in fetal development. In adults, it is replaced by albumin and only present at low levels in the serum. Elevated serum levels of AFP are found in patients with non-seminomatous germ cell tumors, hepatocellular carcinoma and pregnant women due to fetal hepatic production. Lower level elevations sometimes accompany cirrhosis and hepatitis. An elevated serum AFP level is a useful indicator of advanced liver or germ cell tumors or of recurrence of these tumors after treatment. Furthermore, the detection of AFP in tissue sections by immunohistochemistry can aid the pathological diagnosis of unknown primary carcinoma.

*Altered glycolipid and glycoprotein antigens*. Tumor cells often express higher levels or abnormal forms of surface glycoproteins and glycolipids such as mucins, including blood group-related markers, and gangliosides (Baldus et al. 2004). Mucins are high molecular weight glycoproteins with dense O-glycosylation expressed by epithelial tissues. MUC1 is normally expressed on the apical cell surface of glandular and ductal epithelia, including the mammary gland, pancreas, lung and gastrointestinal tract. Tumor-associated MUC1 differs in the pattern of expression and glycosylation profile, and thus, has new carbohydrate and peptide epitopes that can be recognized by the adaptive immune response. Restriction to the apical surface is lost, and MUC1 is expressed at higher levels on the entire cell surface. Differences in O-linked glycosylation are tissue-specific. In general, the core glycans are shorter such that the peptide core is more exposed, and the degree of sialylation increases altering expression of blood group antigens. Membrane-bound MUC1 is involved in cell-cell adhesive and anti-adhesive functions. Adhesion may be mediated by oligosaccharide chains bound to the core protein or the core protein itself. For example, sialyated Lewis blood group antigen sialyl-$Lewis^x$ is recognized by members of the selectin family involved in leukocyte homing, and the core protein which is exposed on tumor cells binds to intercellular adhesion molecule (ICAM)-1. Overexpression of MUC1, as seen in tumor cells, can block cell-cell (such as E-cadherin homophilic interactions) and cell-matrix (such as integrin:extracellular matrix) interactions. All of these interactions may enhance the metastatic potential of tumor cells. MUC1 core protein-specific B cell, CD4+ T cell and CD8+ T cell epitopes have been identified in cancer patients. However, MUC1 also contributes to immune evasion. MUC1 can decrease susceptibility to NK cell and CD8+ T cell cytotoxicity. Tumor-associated MUC1 is unable to efficiently activate CD4+ T cells in vitro. In DCs, the mannose-receptor traps MUC1 in early endosomes resulting in impaired trafficking to late endosomes where class II antigen processing and loading occurs and results in a lower frequency of MUC-1-specific CD4+ T cells. In addition, MUC-1 inhibits the

capacity of DCs to secrete IL-12 and thus skews CD4+ T cell differentation to Th2 cells. MUC1 is shed from the surface and increased in the sera of cancer patients. Several mucins are used in cancer management, such as CA-125 (MUC16) in ovarian cancer and CA-19-9 in ovarian, gastrointestinal and pancreatic cancers. Gangliosides GM2, GD2 and GD3 are glycolipids expressed at high levels in tumors such as melanoma, neuroblastoma and breast cancer. Clinical trials exploring the use of ganglioside-specific monoclonal antibodies and immunization with ganglioside-containing vaccines are ongoing (Chapman et al. 2004; Scott et al. 2005).

### 4.3.3 T Lymphocytes

*CD8+ cytotoxic T lymphocytes*. CD8+ cytotoxic T lymphocytes (CTLs) are principal mediators of tumor killing. Tumor-specific CTLs can be isolated from cancer patients with established tumors. In the case of ovarian and colorectal cancer, CD8+ T lymphocytes have been identified as the lymphocyte population with the TILs that affect survival (Naito et al. 1998; Zhang et al. 2003; Sato et al. 2005a). CD8+ T cell activation requires signals from the TCR, costimulatory molecules and a third signal provided by cytokines IL-12 or IFN-α. In the absence of this third signal, CTLs weakly proliferate, fail to develop effector functions, and are tolerant long term. CD4+ Th cells stimulate DCs to effectively present antigen to CTLs along with costimulation and the appropriate cytokines. After becoming fully activated, CTLs develop activation-induced non-responsiveness, an anergic state characterized by the inability to produce IL-2 needed for further expansion. Nearby antigen-activated CD4+ Th cells can produce IL-2 that can overcome the anergic state and lead to further expansion and development of memory cells. If IL-2 or other proliferative signals are not present, persistent antigen maintains the CTLs in the anergic state. CTL killing is antigen-dependent via the TCR and requires cell-cell contact. Granules containing granzyme and perforin are released at the site of contact. Granzymes are serine proteases that can induce apoptosis in both a caspase-dependent and -independent manner. Perforin forms pores within the plasma membrane of the target cell and facilitates the action of granzymes. CTLs can also induce apoptosis of target cells via membrane-bound death ligands CD95L (FasL) and TRAIL which activate death receptors CD95 (Fas) and TRAILR1 or TRAILR2 on the target cell.

*CD4+ T helper lymphocytes*. Given that the majority of tumors express MHC class I and the cytotoxic effect of CD8+ cells, tumor immunology research initially focused on CTLs. However, it has become clear that CD4+ Th cells are critical for effective and durable antitumor immunity (Knutson and Disis 2005). Tumor-specific CD4+ Th cells are present in cancer patients. CD4+ Th cells are activated by TCR engagement by MHC class II:antigen complex and costimulatory molecules such as CD28 on the T cell interacting with CD80 (B7-1) or CD86 (B7-2) on activated antigen presenting cells. CTLA-4, an inhibitory member of the CD28 family, has a higher affinity for B7-1 and B7-2 and engagement inhibits T cell activation. Th cell differentation is regulated by the environment (cytokines, TCR stimulation, costimulatory molecues) present at the activation of CD4+ T cells. The two major categories are Th1 cells, which produce IFN-γ and TNF, and Th2 cells, which secrete IL-4, IL-5, and IL-13. Th1 cells are responsible for activating cell-mediated immunity and are

especially adept at fighting cancer and intracellular organisms. Th1 cells are also responsible for activating and regulating the development and persistence of CTL. They improve the antigen processing and presenting function of antigen presenting cells, induce production of antibody isotypes that facilitate Fc receptor mediated endocytosis, and activate the killing ability of macrophages through production of NO and ROS. Th1 differentiation depends on high antigen density on the antigen presenting cell, high TCR avidity and the presence of antigen presenting cell-derived IL-12 during antigen presentation. In contrast, Th2 cells are required for optimal antibody production and effective elimination of extracellular pathogens, particularly helminths, but are not effective in anti-tumor activity. Th2 differentiation is promoted by IL-4 and blocked by IL-12. In some cases, tumor-specific CD4+ Th cells possess direct cytotoxic activity using the same mechanisms as CD8+ T cells.

*Regulatory T cells*. An advantage of the adaptive immune response is the extreme diversity and specificity of T and B cell receptors generated by recombination of receptor gene segments. However, the random process also creates T and B cell receptors that recognize self antigens. Since tumors are derived from self, generating an effective anti-tumor response requires avoiding tolerance, mechanisms which delete or suppress the immune response to self antigens. Central tolerance occurs in the thymus during T cell development. T cells that recognize self antigens too strongly are negatively selected and deleted before exiting the thymus. Deletion of high affinity self-reactive lymphocytes during development creates a peripheral repertoire that primarily recognizes non-self antigens. However, autoreactive T cells that escape deletion in the thymus require mechanisms of peripheral tolerance, such as regulatory T (Treg) cells which suppress the function of other immune effector cells through direct cell-cell contact or secretion of cytokines such as IL-10 and TGF-β (discussed below). Two populations of Treg cells exist: natural Treg cells which originate from the thymus and adaptive Treg cells which arise in the peripheral tissues (Wang 2006; Curiel 2007). Although Treg cells have a protective function against auto-immune disease, they have an inhibitory effect on anti-tumor responses. The tumor itself and the tumor microenviroment, including DCs, induce the differentiation of Treg cells by various mechanisms such as TGF-β secretion and expression of B7-H1 (Curiel et al. 2003). In addition, MHC class II-expressing tumors, such as melanoma, may preferentially stimulate CD4+ Treg development given the absence of costimulatory molecule expression on tumor cells. Treg cells were initially described by the expression of CD4 and CD25. However, CD25, the α chain of the high affinity IL-2 receptor, is expressed on activated effector T cells, and its expression does not necessarily correlate with a suppressive phenotype. A better marker of Treg differentiation is the nuclear transcription factor forkhead box P3 (FoxP3). Treg cells may explain why tumors with intact antigen processing and displaying ample tumor antigens are not eradicated by the immune response. Plasmacytoid DCs from a tumor microenvironment can induce T cells to produce the immunosuppresive cytokine IL-10 rather than the protective cytokine IFN-γ induced by plasmacytoid DCs from normal donors (Zou et al. 2001). Myeloid DCs in the tumor microenvironment express the costimulatory molecule B7-H1, which induces T cells to produce IL-10 (Curiel et al. 2003). Elevated proportions of Treg cells in relationship to total CD4+ T cells have been identified in lung, breast, ovarian and melanoma tumors (Woo et al. 2001; Liyanage et al. 2002; Curiel et al. 2004; Viguier et al. 2004), and tumor-

specific Treg cells have been described (Wang et al. 2004; Wang et al. 2005). For example, tumor antigen (LAGE1 and ARTC1)-specific Treg cells have been cloned from TILs in melanoma (Wang et al. 2004; Wang et al. 2005). In ovarian cancer (Jonuleit et al. 2002), breast cancer (Shimizu et al. 1999), and hepatocellular carcinoma (Sutmuller et al. 2001), the number of CD4+ CD25+ FoxP3+ Treg cells inversely correlates with clinical outcome. The frequency of Treg cells is increased in the peripheral blood of patients with CLL and decreases following treatment (Beyer et al. 2005). To overcome immune suppression mediated by Treg cells, some investigators have used denileukin diftitox, a recombinant fusion protein composed of a diphtheria toxin fragment coupled to IL-2, to deplete Treg cells. Denileukin diftitox targets the cytocidal action of diphtheria toxin to cells expressing CD25. It is approved for the treatment cutaneous T cell lymphoma. In addition to directly killing malignant T cells expressing CD25, there may also be a benefit to eliminating Treg cells. Denileukin diftitox decreases the number of Treg cells and improves immunity in individuals with renal cell carcinoma (Dannull et al. 2005) and melanoma (Mahnke et al. 2007). In contrast, another group observed no reduction in the number of Tregs or their function in melanoma patients receiving denileukin diftitox (Attia et al. 2005). When considering the use of denileukin diftitox in cancers that do not express CD25, the drug will target both Treg cells and activated effector T cells with anti-tumor activity. Furthermore, TLR8 ligands have been shown to have a direct effect on Treg cells and reverse the suppressive phenotype in a mouse model (Peng et al. 2005). Of interest, imiquimod, a topical agent approved for the treatment of genital warts, actinic keratosis and superficial basal cell carcinoma, is a TLR7 and TLR8 agonist. In addition to acting on antigen presenting cells to improve antigen processing and presentation and thus improving T cell activation, TLR8 stimulation also decreases the suppressive function of Treg cells.

### 4.3.4 B Lymphocytes

Although not the dominant effector in the anti-tumor immune response, B cells generate tumor-specific antibodies, and tumor-specific antibodies have been identified in many cancers (Cassard et al. 2006). These antibodies can coat tumor cells and trigger tumor cell destruction by antibody-dependent cell-mediated cytotoxicity or by activating complement. In antibody-dependent cell-mediated cytotoxicity, antibody-coated target cells engage the Fc receptors on NK cells, macrophages and neutrophils and trigger cytokine secretion and release of toxic granules which mediate killing. In complement-dependent cytotoxicity, the antibody-coated target activates the complement pathway and results in the formation of the membrane attack complex, which forms a pore in the target cell causing death. Antibodies also increase the afferent arm of adaptive immunity. Fc receptor engagement by antibody-coated targets mediates activation of phagocytes and improved efficacy of phagocytosis and antigen processing and presentation. The therapeutic efficacy of monoclonal antibodies, such as rituximab and trastuzumab (discussed above) supports a role for antibodies in anti-tumor defenses. In addition, induction of

antibodies by vaccination can prevent infection by oncogenic viruses, and thus, prevent virally-induced tumors (see below). Interestingly, in some mouse models B cells can inhibit the anti-tumor immune response through diminished IFN-γ production, which may result from IL-10 secretion by B cells (Shah et al. 2005; Inoue et al. 2006). IL-10, is a cytokine that downregulates immune responses by decreasing macrophage and DC production of IL-12 and thus decreasing IFN-γ production by NK cells and T cells. IL-10 also inhibits expression of costimulatory molecules and MHC class II expression on antigen presenting cells.

### 4.3.5 Cytokines

*Interleukin-2*. The principal function of IL-2 is to promote the growth, survival and differentiation of T cells. IL-2 is produced by CD4+ T cells and can serve as an autocrine or paracrine growth factor (Waldmann 2006). The high affinity IL-2 receptor (IL-2R) is expressed on antigen-activated T cells, Treg cells and malignant cells of various B and T cell malignancies. Thus, IL-2 supports the survival, proliferation, differentiation of antigen-activated T cells as well as the survival and function of Treg cells. IL-2 also functions to generate peripheral tolerance through activation-induced cell death, a mechanism of eliminating self-reactive, peripheral T cells. IL-2 induces the proliferation of B cells and immunoglobulin synthesis. Furthermore, it stimulates the generation of CD8+ CTLs and promotes the differentiation, proliferation and activation of NK cells. The anti-tumor activity of IL-2 likely stems from stimulating the proliferation and activity of NK cells and CD8+ CTLs.

Recombinant IL-2 (aldesleukin) is used in treatment of metastatic melanoma and metastatic renal cell carcinoma as well as some settings of acute myelogenous leukemia, non-Hodgkin lymphoma and cutaneous T cell lymphoma. In metastatic melanoma, IL-2 alone results in an objective response rate of 5–27% and a complete response rate of 0–4% (Petrella et al. 2007). When administered together with lymphokine activated killer cells, the complete response rate increases to 0–11% with the possibility of achieving long term response (median 27 months) (Petrella et al. 2007). Systemic use of IL-2 is limited by capillary leak syndrome.

*Interleukin-15*. The receptor for IL-15 shares the β and γ subunits with the IL-2R, but has a unique α subunit (Waldmann 2006). Therefore, these cytokines share several functions. Like IL-2, IL-15 stimulates the proliferation of T cells, induces the generation of CD8+ CTLs, facilitates the proliferation of B cells and immunoglobulin synthesis and induces the generation and survival of NK cells. In contrast to IL-2, IL-15 inhibits activation-induced cell death. Unique to IL-15, it supports the survival of CD8+ memory T cells. IL-15 has the potential to be a better candidate than IL-2 for inhibiting tumor growth. In the presence of IL-2, CD8+ CTLs might recognize the tumor antigen as self and undergo activation-induced cell death, or the anti-tumor immune response may be inhibited by IL-2-dependent Treg cells. In contrast, IL-15 has the ability to activate T cells and NK cells and has the advantage of inhibiting activation-induced cell death and promoting

the persistence of CD8+ memory T cells. IL-15 is effective in preventing tumor growth in mouse models and in producing long-lasting cellular immunity.

*Interferon*-γ. IFN-γ, also called type II IFN, plays a critical role in augmenting both innate and adaptive immunity (Stark et al. 1998). IFN-γ is produced by NK cells in response to IL-12 or by CD4+ Th1 cells and CD8+ T cells in response to antigen activation. IFN-γ activates macrophages to generate NO and ROS and improve killing of intracellular organisms. IFN-γ promotes the differentiation of naïve CD4+ T cells into Th1 cells generating effective cell-mediated immunity for attack of intracellular microbes and cancer cells. IFN-γ promotes Th1 differentiation by enhancing synthesis of IL-12 by antigen presenting cells and blocking differentiation of Th2 cells by inhibiting production of IL-4. Similar to type I IFNs, IFN-γ enhances expression of MHC class I. IFN-γ also induces expression of proteosome subunits that make up the immuno-proteosome in antigen presenting cells, as well as TAP subunits further enhancing MHC class I-restricted processing and presenting and thus, promoting recognition and killing of malignant or infected cells by CD8+ CTLs. In addition, IFN-γ uniquely induces MHC class II expression on a wide variety of cell types via the class II transactivating (CIITA) factor. Together with increased MHC expression, IFN-γ increases costimulatory molecule expression enhancing antigen processing and T cell activation. IFN-γ promotes B cell class switching to complement fixing IgG subclasses and inhibits switching to IgE. IFN-γ promotes leukocyte trafficking to sites of inflammation and cancer by increasing the expression of adhesion molecules and chemokines involved in leukocyte homing.

IFN-γ promotes an anti-tumor immune response when expressed in the tumor microenvironment, but has limited efficacy as a systemic agent (Tannenbaum and Hamilton 2000). Tumor cells transfected with IFN-γ have a poor capacity to form tumors and induce potent anti-tumor immunity. Systemic delivery of IL-12 causes a strong anti-tumor effect by inducing IFN-γ expression in the tumor microenvironment that can not be achieved with systemic treatment using IFN-γ even at high doses. The anti-tumor response often depends on the tumor cells being responsive to IFN-γ. Indeed, under the selective pressure of the immune response as many as one third of human tumors have lost sensitivity to IFN-γ through loss of IFN-γ receptor chains, IFN-γ signaling pathway kinases or transcription factors. Although IFN-γ can display pro-apoptotic and anti-proliferative activity on tumor cells, its primary anti-tumor effect derives from its immunomodulatory properties discussed above.

*Transforming growth factor*-β. Whereas cytokines like IL-2 and IFN-γ promote the anti-tumor response, TGF-β is involved in negatively regulating immune responses and can be produced in the tumor microenvironment to contribute to immune evasion (Li et al. 2006). TGF-β is produced by a variety of cells including activated T cells, macrophages, DCs, NK cells, tumor cells and some Treg cells. TGF-β maintains peripheral tolerance via regulation of lymphocyte proliferation, differentiation and survival. It exerts an anti-proliferative effect on T cells by inhibiting expression of T cell growth factor IL-2. In addition, TGF-β blocks expression of cell cycle promoting factors, including c-myc, cyclin D2, cyclin E, and cyclin-dependent kinase 4, and upregulates cyclin dependent kinase inhibitors. TGF-β decreases differentiation of naïve CD4+ T cells into Th1 and Th2 cells as evidenced by decreased T-bet (Th1-specific transcription factor) and GATA-3 (Th2-specific transcription

factor), respectively. TGF-β also inhibits the differentiation of CD8+ T cells via decreased T-bet expression and reduces the cytolytic function of CD8+ T cells through inhibition of perforin and FasL expression. In contrast, TGF-β in the absence of pro-inflammatory cytokines promotes the differentiation of CD4+ T cells into Treg cells, which actively suppress immune responses and maintain immunogenic tolerance. TGF-β's regulatory activity is modulated by the state of cell differentiation and the presence of inflammatory cytokines and costimulatory molecules. In the absence of CD28 costimulation, TGF-β inhibits TCR-stimulated proliferation of naïve T cells. Since tumor cells express MHC class I but tend to lack costimulatory molecules, TGF-β blunts the anti-tumor response. Conversely, in the presence of CD28 costimulation, TGF-β promotes T cell proliferation and inhibits apoptosis. In addition, TGF-β promotes the survival of effector memory T cells and inhibits Fas-induced T cell apoptosis through decreased c-myc expression and decreased levels of FasL. TGF-β blocks B cell activation and class switching, except for IgA, and induces apoptosis of naïve B cells.

TGF-β controls initiation and resolution of inflammatory responses through regulation of chemotaxis, activation and survival of lymphocytes, NK cells, DCs, macrophages and neutrophils (Li et al. 2006). TGF-β is a potent inhibitor of NK cell function by diminishing NK cell cytolytic activity and IFN-γ production and promoting NK cell homeostasis. TGF-β antagonizes IL-12-induced IFN-γ production that is essential to Th1 differentiation. TGF-β blocks the cytolytic function of NK cells by inhibiting expression of NKp30 and NKG2D receptors. TGF-β inhibits DC maturation, and in the presence of TGF-β DCs have decreased MHC class II and costimulatory molecule cell surface expression. On the other hand, TGF-β is required for the differentiation and survival of Langerhans cells, resident DCs within the epidermis. TGF-β acts as a chemoattractant for monocytes, induces the expression of adhesion molecules such as LFA-1 on monocytes, and induces production of MMPs that facilitate transmigration. Once monocytes differentiate into tissue macrophages, TGF-β is mostly inhibitory. It downregulates scavenger receptors involved in phagocytosis, downregulates expression of FcγRI and FcγRIII which results in decreased phagocytosis of IgG-coated particles, decreases expression of inflammatory mediators such as TNF-α and MMP-12 as well as chemokines MIP-1α and MIP-2, downregulates production of NO and ROS, and blocks IFN-γ-induced expression of MHC class II via attenuation of CIITA.

Tumors that produce increased TGF-β or promote TGF-β production by surrounding cells advance tumor progression and allow immune evasion (Li et al. 2006). In mouse models, overexpression of TGF-β suppresses the anti-tumor immune response, and inhibition of TGF-β expression is associated with decreased tumor growth. TGF-β's inhibition of the anti-tumor immune response is due to decreased NKG2D expression on NK and CD8+ T cell, decreased MICA expression on tumor cells, inhibition of CD8+ CTLs, and induction of Treg differentiation. Elevated levels of TGF-β are associated with down-modulation of NKG2D surface expression on NK cells in lung and colorectal cancer patients (Lee et al. 2004). As discussed earlier, the frequency of TGF-β induced Treg cells is increased in cancer patients and portends a poorer prognosis. Additionally, Smad3 protein, an intracellular transcription factor activated by phosphorylation by the TGF-β family of serine/threonine kinase receptors, is absent or reduced in several cases of human

T cell acute lymphoblastic leukemia suggesting the importance of TGF-β signaling in preventing lymphoproliferative disease.

## 4.4 Immunotherapy for Cancer Prevention

Much cancer immunotherapy research has focused on the difficult challenge of treating metastatic disease. A review of 1,306 patients with solid tumors treated with immunotherapy revealed an overall objective response rate of 3.3% underscoring the important need for new strategies in immunotherapy (Rosenberg et al. 2004). Analogous to the situation with infectious disease, it is immunologically more feasible to use vaccines to boost the immune response and prevent cancer than to use vaccines to treat advanced disease. Therefore, the prospect of developing of vaccines to prevent cancer is exciting. Tumors that have tumor-specific antigens shared by the vast majority of individuals with that tumor are candidates for preventive vaccines. Virally-induced tumors are primary examples of tumors that may be prevented by vaccination.

HPV types 16 and 18 cause approximately 68% of cervical squamous cell cancer and 83% of cervical adenocarcinoma (FUTURE II Study Group 2007; Markowitz et al. 2007). A large percentage of anogenital cancers involving the anus, vulva, vagina, penis and a smaller percentage of oral cavity and pharyngeal cancers are also attributable to HPV, especially HPV type 16. In June 2006, the quadrivalent HPV vaccine was FDA-approved for the prevention of cervical cancer, cervical cancer precursors, vaginal and vulvar cancer precursors, and anogenital warts caused by HPV types 6, 11, 16 and 18 in women aged 9–26 years. The vaccine prevents the development of precancerous lesions of the cervix, vagina and vulva; vaccine efficacy was 100% for prevention of HPV type 16 and 18 related cervical intraepithelial neoplasia grade 2 or 3 (CIN 2/3), adenocarcinoma in situ (AIS), vulvar intraepithelial neoplasia grade 2 or 3 (VIN 2/3), and vaginal intraepithelial neoplasia grade 2 or 3 (VaIN 2/3). Of note, there was no evidence of cancer prevention from disease caused by HPV types for which the subject was polymerase chain reaction (PCR) positive prior to vaccination.

In addition, hepatitis B virus (HBV) vaccination has the potential to prevent HBV-related hepatocellular carcinoma. Since the introduction of the HBV vaccine in 1982, a comprehensive strategy to eliminate HBV transmission and prevent its consequences has been implemented in the United States. This strategy includes universal vaccination of neonates, routine screening of pregnant woman and post-exposure prophylaxis for neonates, vaccination of children and adolescents not previously vaccinated, and vaccination of at risk adults (Mast et al. 2006). Approximately 50% of hepatocellular carcinoma cases are associated with HBV, and the association is stronger in children, who are more likely to have chronic infection than adults. In Taiwan, the incidence of childhood hepatocellular carcinoma has significantly decreased since the institution of a universal HBV vaccination program (Chang et al. 1997). Since the incidence of hepatocellular carcinoma peaks in the sixth decade of life, it will take more than 40 years for the full impact of HBV vaccination on the prevention of hepatocellular carcinoma to be observed.

4

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# Hereditary Risk for Cancer

# 5

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Understanding the role of heredity in cancer is a key component of cancer prevention. Individuals at a high risk for cancer due to hereditary predisposition can now be identified through a comprehensive cancer risk assessment evaluation and/or cancer genetic testing. Identification of individual's positive for mutations or those at high risk prior to cancer occurrence provides an opportunity to intervene with prevention and screening strategies documented to reduce cancer incidence or the mortality from cancer occurrence. Currently, there are over 200 hereditary cancer syndromes described in the literature (Schneider 2001). The scope of this chapter is not to describe all hereditary cancer syndromes, but to provide the tools to identify a hereditary cancer syndrome in a family and describe the process individuals and families undergo to determine a hereditary susceptibility towards cancer. The more common hereditary breast, ovarian and colon cancer syndromes will be described along with recommended screening and surveillance, prophylactic and chemoprevention options currently available to those at increased risk.

## 5.1 Cancer as a Genetic Disorder

Cancer is the outcome of an evolution of genetic mutations and epigenetic effects on our DNA over time. Therefore, cancer is a genetic disease however, not all cancer is hereditary. To understand the hereditary predisposition to cancer, it is first important to provide a basic review of the molecular genetics involved in cancer progression.

### 5.1.1 Molecular Genetics

A gene is the basic unit of heredity, encoded in the deoxyribonucleic acid (DNA) that make up our 46 chromosomes found in the nucleus of our cells. Our chromosomes travel

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in pairs. The first 22 pairs are the autosomes. Genes located on an autosomal chromosome are present in both males and females and are therefore inherited in an autosomal inheritance pattern. The 23rd chromosome pair make up the sex chromosomes. Males have an X and a Y chromosome. Females carry two X chromosomes. Genes located on the X and the Y chromosomes are sex-linked and not identified in hereditary cancer.

Genetic information is inherited from parents through the chromosomes. Half of the total genetic information comes from each parent's chromosomes (23 from each parent). The germline cells found in ovum and sperm are the only cells that do not return to the original total number of 46 following mitosis.

Mutations can be classified in three groups: *genome mutations* are those that change the total number of chromosomes in the cell, *chromosomal mutations* change the structure of the individual chromosomes and *genetic mutations* change our genetic sequence (Nussbaum et al. 2001). For the purpose of this discussion on hereditary cancer, the focus will be only on genetic mutations as they apply to hereditary cancer syndromes.

Mutations can occur in the germline cells and in somatic cells. The germline mutations are the only mutations passed from generation to generation and are therefore responsible for hereditary predisposition to cancer.

### 5.1.2 Tumor Suppressor Genes

The majority of hereditary cancer syndromes are caused by mutations in the tumor suppressor genes in the germline cells (Schneider 2001). The function of the proteins expressed from tumor suppressor genes is to negatively regulate cellular growth, especially in damaged cells (Schneider 2001). Tumor suppressor genes are inherited dominantly in hereditary cancer syndromes, but behave recessively on the cellular level (Franks and Teich 1997). When an individual inherits a mutation on one copy of a tumor suppressor gene, the protein produced from the working gene will maintain cell growth and suppress cancer (Schneider 2001). If this individual were to lose the working copy of the same gene, tumor growth can occur in the target cell. Mutations in tumor suppressor genes do not always begin with a germline mutation followed by a somatic mutation. Cancer may also result from two somatic mutations. This form of cancer initiation is thought to represent the majority of sporadic cancer occurrences (Schneider 2001).

Alfred Knudson advanced the understanding of cancer as a hereditary disease with his model of retinoblastoma. This model explained cancer as a two-hit progression of cancer. He compared the inherited form of retinoblastoma, occurring in nine out of every ten children with an inherited mutation, to the sporadic form that occurs in one of every 20,000 children in the US (Knudson 1971). The children with the inherited mutation were much more likely to be affected with cancer because they were born with "one hit" or germline mutation versus the sporadic cases requiring two acquired mutations in the somatic cells.

Vogelstein furthered the two-hit theory with his model of carcinogenesis first described in 1990 (Fearon and Vogelstein 1990). Vogelstein's model described multiple hits occurring in normal colon cells causing increasingly more mutations and resulting in carcinoma.

His model was based on the adenomatous polyposis coli (APC) tumor suppressor gene which is mutated in the germ-line cells in inherited cases of familial adenomatous polyposis (FAP). The first mutation in the APC gene, whether it be somatic or hereditary, can be thought of as "the gate-keeper" gene (Schneider 2001). A mutation in the gate-keeper gene allows for additional gene mutations, such as activation of the K-RAS oncogene and loss of other tumor suppressor genes to occur more readily. The succession of genetic mutations and the order in which these mutations occur is important in determining stages of development of adenomas.

Understanding changes in the gene on the molecular level will eventually lead to better treatment protocols and prevention recommendations tailored to an individual's genetic changes (Schneider 2001).

### 5.1.3 Oncogenes

There are relatively few hereditary cancer syndromes which occur as a result of oncogenes (Schneider 2001). Oncogenes arise from proto-oncogenes which are responsible for regulating the cell's signaling pathway. A mutation in the proto-oncogene activates the oncogene causing either increased expression of the proto-oncogene protein or a change in the structure and function of the proto-oncogene's protein (Schneider 2001). Most mutations occur on the somatic level and behave dominantly, requiring only one mutation to cause abnormal cell growth. As is described in Vogelstein's cancer progression model, more than one activated oncogene in a target cell or cells is necessary for cancer to occur.

### 5.1.4 DNA Repair Genes

The purpose of DNA repair genes is to identify and repair DNA errors made in the nucleotide sequence during replication. DNA repair involves several steps, including identifying the error in the DNA strand, gathering necessary proteins for repair, incision of the DNA and excision of the nucleotide sequence that is erroneous, new synthesis of the correct nucleotides and reattachment of the correct sequence (Schneider 2001). Mutations in the genes involved in the repair process can lead to accumulations of DNA errors within a cell. A defective DNA repair gene has a secondary effect on cancer progression, whereas mutations in oncogenes and tumor suppressor genes cause a primary effect (Schneider 2001).

Several DNA repair genes, called mismatch repair genes (MMR) have been implicated in hereditary nonpolyposis colorectal cancer syndrome (HNPCC), which is described later. MMR genes work in the cell to repair errors that occur during replication. In cells with mutated mismatch repair genes, a phenomenon called microsatellite instability (MSI) occurs. MSI refers to the instability of the microsatellite sequences, short repeats of six or less base pairs, causing long strings of the same repeated sequence. The presence of MSI results in the progression of more somatic mutations in the cell (Offit 1997).

## 5.2 Epigenetic Mechanisms

Epigenetic effects imply a nongenetic influence on the DNA that changes gene expression through the activation or silencing of growth regulatory genes. Genomic imprinting and methylation are two examples of epigenetic mechanisms causing gene silencing. When growth regulation genes are silenced by this mechanism, cells may receive a potential hit (Schneider 2001). Epigenetic effects are important because these changes may be the most common alteration seen in human cancer. Because epigenetic mechanisms are not thought to be the result of DNA changes, the process may be reversible, leading to better targeted treatment options for patients in the future (Schneider 2001).

Cancer occurs as a multi-step model in which a succession of events, genetic mutations, environmental agents, epigenetic agents, leads to cancer progression. Learning the molecular changes in cells and following the sequential progression of cancer may allow the development of effective interventions that will stop cancer progression on the molecular level.

## 5.3 Cancer as a Hereditary Disease

The majority of the 200 hereditary cancer syndromes described in the literature are rare and account for between 5 and 10% of all cancers. Identifying a sporadic occurrence of cancer versus a familial or hereditary form is the first step in locating patients at higher cancer risk, and thus providing appropriate screening and prevention recommendations. Differentiation between sporadic and hereditary cancer is often obvious while elucidating a hereditary versus familial form of cancer can be subtle. The latter requires familiarity with the hereditary cancer syndromes in addition to obtaining a well documented and detailed family history from the patient.

A sporadic cancer family can be described by a single occurrence of cancer, typically at a later age of onset. For example, the proband (the affected family member from which the family is ascertained) is a 70 year old woman recently diagnosed with breast cancer. There are no other incidences of cancer in first degree relatives (mother and father, sisters and brothers) or second degree relatives (grandparents, aunts and uncles, nieces and nephews). Family history is also negative for cancer in the third degree relatives (cousins and great aunts and uncles). The cancer described in this family would be classified as a sporadic incidence of cancer with no concern of inherited susceptibility.

Familial cancer syndromes are more difficult to differentiate from hereditary cancer due in large part to the variability in gene expression exhibited by hereditary cancer syndromes. The typical family history reported for a potential familial form of cancer is described with at least two relatives who are affected with similar cancers or two relatives

with distinct cancers. The ages of onset in familial cancer tend to occur at a later age. For example, the proband is diagnosed with breast cancer at age 68. The proband's mother has never been affected with cancer, but a maternal aunt was diagnosed at a later age, early 1980s, also with breast cancer. This family history is not as compelling for hereditary cancer because the ages of onset are closer to expected age of diagnosis for sporadic cancers. In addition, and more importantly, the cancer occurrences do not follow an autosomal dominant inheritance pattern. Another example of possible familial cancer is the following: a proband is diagnosed with prostate cancer at age 75 and reports a maternal grandfather affected with colon cancer at age 72. Despite two cancer occurrence in this family, the ages of onset are later and autosomal dominant inheritance is not demonstrated.

Two hallmarks of hereditary cancer are early ages of onset and occurrence in at least two successive generations. Hereditary cancer may occur up to 20 years younger than the expected age for the specified cancer. Individuals who carry an autosomal dominant mutation for hereditary breast and ovarian cancer are at an increased risk for bilateral cancers, as well as for a second occurrence of cancer. Most hereditary cancer syndromes are also associated with increased risk for multiple primary cancers. These factors contribute to the importance of identifying at-risk individuals so that recommendations can be given to prevent cancer from occurring or to reduce morbidity and mortality from cancer. Perhaps the most important characteristic of hereditary cancer syndromes is the potential for increased risk to the relatives of the affected individual who is found to carry a hereditary predisposition gene.

Because the hereditary cancer genes are located on the autosomal chromosomes, both males and females may inherit these cancer susceptibility genes. Expression of disease after inheriting the gene will differ between men and women depending on the types of cancer exhibited in the hereditary cancer syndrome as well as the type of mutation inherited. For an autosomal dominantly inherited hereditary cancer syndrome, there is a 50% risk of passing down a genetic mutation from the carrier to an offspring. In a typical family history with a hereditary cancer gene mutation there tends to be at least one affected relative in each generation. However, this is not consistently demonstrated. There are many families in which an individual carries one of the gene mutations for an autosomal dominant cancer syndrome, yet they do not express the phenotype of the mutation and are not affected with cancer. This phenomenon is observed because hereditary cancer syndromes demonstrate variable expression of the disease phenotype. Another example of variable expressivity is seen with the lack of similarity among the ages of diagnosis between relatives with the same cancer. In addition, most hereditary cancer genes exhibit incomplete penetrance. Penetrance describes an all or none effect of gene expression. Individuals will either have signs and symptoms of the disease or they will not.

There are only a few hereditary cancer syndromes inherited in an autosomal recessive manner. Autosomal recessive disorders occur when an individual inherits the same genetic mutation on each chromosome. A carrier for an autosomal recessive gene is not affected with the disease. When parents carry the same autosomal recessive gene mutation, the risk to have an affected child is one in four or 25%.

## 5.4 Common Hereditary Cancer Syndromes

### 5.4.1 Hereditary Breast and Ovarian Cancer

Genetic predisposition provides one of the most significant risk factors for women to develop either breast or ovarian cancer or both cancers. Of all the genetic predisposition genes, the breast and ovarian cancer genes provide one of the highest risks for increased cancer susceptibility. Genetic testing became available for the breast and ovarian cancer syndrome in the mid 1990s. Currently, there are two genes described for the hereditary breast and ovarian cancer syndrome. The first gene described, BRCA1, located on chromosome 17q21 was identified in 1989 and sequenced in 1994 (Hall et al. 1990; Miki et al. 1994). The second gene, BRCA2, located on chromosome 13q12 was sequenced in 1995 (Wooster et al. 1995).

The BRCA1 and BRCA2 genes are thought to account for approximately 80–90% of all hereditary breast and ovarian cancers (Thull and Vogel 2004). Similar to other hereditary cancers, between 5 and 10% of all breast cancers and up to 10% of all ovarian cancers are thought to be due to inherited susceptibility (Malander et al. 2004).

The incidence of a BRCA1 or BRCA2 gene mutation in individuals of Northern European descent is between 1 in 800 and 1 in 2,500 (Schneider 2001). In North America, 1 in every 300–500 people is estimated to harbor a germline BRCA mutation (Nelson et al. 2005). Several founder mutations specific to geographically isolated populations have been identified in hereditary breast and ovarian cancer. The most well described founder mutation is found in the Ashkenazi Jewish population in which the carrier frequency for a BRCA1 or BRCA2 gene mutation is 1 in 40 or approximately 2.5% (Tonin et al. 1996). Other founder mutations have been identified in the Dutch, Icelandic, French Canadian and other populations (Johannesdottir et al. 1996; Tonin et al. 1996, 1998; Peelen et al. 1997; Petrij-Bosch et al. 1997).

The BRCA1 gene is large, with 24 exons, encoding a protein of 1,863 amino acids and works as a tumor suppressor gene (Miki et al. 1994). The protein function of a normal BRCA1 gene is to recognize DNA damage (Couch et al. 1996). Referring back to the model of carcinogenesis, the inherited mutation of one BRCA1 gene leads to increased susceptibility to mutations in the remaining somatic copy of the BRCA1 gene beginning the pathway to carcinogenesis.

The 800 hereditary mutations identified on the BRCA1 gene are found throughout the gene sequence. The majority of these mutations lead to premature protein termination and are frameshift or nonsense mutations (Barnes-Kedar and Plon 2002). Testing for a BRCA1 gene mutation is complex. This complexity is due in part to the fact that the majority of gene mutations described to date have been identified as private mutations which are unique to each family undergoing testing (Tirkkonen et al. 1997). An exception to these private mutations is seen with the founder mutations. The BRCA1 contains two of the three founder mutations for the Ashkenazi Jewish population. Approximately 1% of the Ashkenazi Jewish population carry the mutation, 185delAG, and 0.1% of the Ashkenazi population carry the founder mutation, 5382insC (Peelen et al. 1997). Tumors

from BRCA1 mutation carriers are predominantly of basal subtype, which corresponds to the triple-negative phenotype (i.e., negative for ER, PR, and HER2 expression) (Turner and Reis-Filho 2006). This tumor subtype underlies the poor prognosis of breast cancer in BRCA1 mutation carriers.

The BRCA2 gene is even larger than the BRCA1 gene with 27 exons, encoding a protein of 3,418 amino acids (Tirkkonen et al. 1997). The BRCA2 gene, like BRCA1, is also a tumor suppressor gene with protein product is also involved in DNA repair (Couch et al. 1996). There have been approximately 100 mutations reported in the BRCA2 gene and these mutations also play a role in premature chain termination (Tirkkonen et al. 1997). Unique to the BRCA2 gene is a region identified as the ovarian cancer cluster region (OCCR). Mutations found in this region confer lower risks for women to develop breast cancer and increased risks for developing ovarian cancer (Thompson et al. 2001). There is one Ashkenazi Jewish founder mutation on BRCA2, 6174delT. This mutation is seen in approximately 1% of individuals of Ashkenazi Jewish descent (Peelen et al. 1997). Most tumors that develop in BRCA2 carriers are of luminal subtype (i.e., positive for ER and PR and negative for Her2 expression) (Bane et al. 2007). The luminal subtype has a good prognosis. Interestingly, neither the BRCA1 nor BRCA2 genes exhibit mutations in truly sporadic breast cancer.

In addition to increased susceptibility to breast and ovarian cancer, mutations in the BRCA1 and BRCA2 genes confer risks for other cancers as well. While this is still controversial, early studies report an increased risk for colon cancer in carriers of the BRCA1 gene mutations (Ford et al. 1994). Follow-up studies failed to duplicate these risks (Peelen et al. 2000). Males who carry the BRCA1 gene mutation may also have an increased risk for prostate cancer (Liede et al. 2004). It is likely that men and women with a BRCA1 or BRCA2 mutation have an increased risk for developing cancer of the pancreas, or stomach, or, for women, primary peritoneal serous carcinoma; however the risk is rather small (Nelson et al. 2005).

Male breast cancer has also been correlated with a BRCA2 gene mutation. There are a variety of other cancers also associated with the BRCA2 gene mutations, including prostate, pancreatic, gallbladder, bile duct, stomach and malignant melanoma (Risch et al. 2001).

Current estimation of cancer risks for carriers of a BRCA1 or BRCA2 mutation indicate the lifetime risk for breast cancer is between 50 and 87%. The risks for ovarian cancer differ between the BRCA1 and the BRCA2 gene mutations.

The risks for ovarian cancer in women who carry a BRCA1 gene are estimated to be 15–40%. The risks for ovarian cancer in women who carry a BRCA2 gene are 14–27% (Struewing et al. 1997; Risch et al. 2001).

The risk of breast cancer in men who carry a BRCA2 mutation is between 2.8 and 6.3% (Thompson et al. 2001). The risk of prostate cancer in men who carry a BRCA1 gene mutation is between 8 and 16% based on one study (Ford et al. 1994). The risk of prostate cancer in men who carry the BRCA2 gene mutation is between 7 and 16% and had been estimated to be as high as 20% before age 80 with the relative risk greater for men younger than 65 (Struewing et al. 1997; 1999).

The risks for colon cancer in men and women who carry a BRCA1 gene mutation is 6% based on data reported from the Breast Cancer Linkage Consortium. Pancreatic cancer was also found in studies to be increased in BRCA2 mutation carriers with a 2.1% cumulative risk by age 70 (1999).

The wide range of cancer risks described from mutations in the BRCA1 and BRCA2 genes can be explained by inadequate study designs with potential for biased ascertainment as well as variable expressivity of the gene, environmental factors and other gene modifiers. A recent report on risks for breast and ovarian cancer in Ashkenazi Jewish women with inherited BRCA1 and BRCA2 mutations identified physical exercise and lack of obesity in adolescent as modifiable risk factors, delaying the age of breast cancer onset (King et al. 2003). Further studies are underway to determine effects of environmental factors and other modifier genes on the expression of these gene mutations.

### 5.4.2 Cowden Syndrome

There are a handful of other hereditary cancer syndromes that increase susceptibility to breast cancer. These syndromes are typically much rarer in the general population and include an increased susceptibility to a variety of other cancers as well. Cowden syndrome, also called multiple hamartoma syndrome, accounts for less than 1% of hereditary breast and ovarian cancer. The incidence of Cowden syndrome is approximately 1 in every 200,000–250,000 individuals (Schneider 2001). The incidence of this syndrome is likely underreported given the variety of unusual findings. Cowden syndrome is inherited in an autosomal dominant pattern conferring a 50% risk to the offspring of an affected male or female. The gene for Cowden syndrome is located on chromosome 10q23 and penetrance of the gene may be as high as 100% (Nelen et al. 1996). The gene mutated in Cowden syndrome, PTEN, is a tumor suppressor gene, only nine exons in length. The role of the protein product is to control cell cycle arrest and apoptosis (Schneider 2001). As many as 80% of individuals with clinic findings suggestive of Cowden syndrome will have gene mutation identified with clinical testing (Eng 2000).

The clinical features of Cowden syndrome are unique from hereditary breast and ovarian cancer due to the physical findings associated with this syndrome. The pathognomonic features of Cowden syndrome are facial trichilemmomas, acral keratoses, oral papillomatous papules and mucosal lesions (Eng 2000). Major criteria used to establish a clinical diagnosis include breast cancer, thyroid cancer, especially papillary carcinoma, macrocephaly, Lhermitte–Duclos disease or cerebral dysplastic gangliocytoma, and endometrial cancer. The minor criteria include other thyroid lesions, mental retardation, gastrointestinal hamartomas, fibrocystic breasts, lipomas or fibromas, genitourinary tumors (renal cell carcinoma, uterine fibroids) and genitourinary malformations. Diagnosis is confirmed clinically when the affected proband presents with either six facial papules listed in the pathognomonic criteria, two major criteria that must include macrocephaly or Lhermitte–Duclos disease, one major criteria and three minor criteria, or four minor criteria (Eng 2000).

The lifetime risk for breast cancer in women who are identified with Cowden syndrome is between 25 and 50%, and at least 75% of women with Cowden syndrome have benign breast disease (fibroadenoma and fibrocystic breasts) (Brownstein et al. 1978). Male breast cancer has also been identified in men who carry a PTEN mutation, but the specific risks are not documented (Fackenthal et al. 2001). Endometrial cancer is also considered in Cowden syndrome with risks reported between 5 and 10% (Eng 2000). Lifetime

risk for thyroid cancer (usually follicular, rarely papillary, and never medullary) is around 10% (Eng 2000).

### 5.4.3 Li Fraumeni Syndrome

Li Fraumeni syndrome (LFS) is another rare syndrome conferring increased risk for breast cancer in affected women. The incidence of LFS is unknown. LFS accounts for less than 1% of hereditary breast cancer. The inheritance of LFS, similar to the other hereditary breast cancer syndromes, is autosomal dominant. The gene is located on chromosomes 17p13.1 (Levine 1997). Individuals who meet diagnostic criteria for LFS could elect to undergo genetic testing. The majority of families who meet diagnostic criteria for LFS are found to carry an identifiable genetic mutation. The gene for LFS, p53, is also a tumor suppressor gene and is involved in cell repair, the apoptosis pathway and maintaining genomic stability (Schneider 2001). A mutation in the hCHK2 gene has been identified in some families with LFS (Barnes-Kedar and Plon 2002).

The clinical characteristics of LFS include early onset of breast cancer (before the 40th decade of life), soft tissue sarcomas, primary brain tumors, adrenocortical carcinomas and acute leukemias (Malkin et al. 1990). Other cancers associated with LFS include malignancies in the stomach, colon and lung as well as childhood neuroblastomas and increased risk of melanoma (Schneider 2001). Clinical diagnosis is considered when the proband reports being diagnosed with an isolated sarcoma before age 40. In addition to reporting one first degree relative and another first or second degree relative affected with associated LFS tumors, clinical diagnosis of LFS is considered when cancer is diagnosed before age 45 or a sarcoma is diagnosed at any age (Garber et al. 1991).

The risk of all cancers in individuals with LFS is estimated to be 50% by age 30 and 90% by age 70. Mutations in p53 also place a carrier at a 50% risk of a second primary tumor (Schneider 2001; Thull and Vogel 2004).

### 5.4.4 CHEK2

At this point, a BRCA3 gene has not been identified. Instead, genes with low to moderate penetrance have been proposed to play a role in HBOC. CHEK2 is the most important breast cancer susceptibility gene identified since BRCA2. CHEK2 acts as a tumor suppressor gene and is a cell cycle regulator. It is located on chromosome 22 at position 22q12.1. As mentioned previously, CHEK2 mutations have been identified in several families with cancer characteristics of Li Fraumeni Syndrome (LFS). Researchers are uncertain whether CHEK2 mutations actually cause LFS or are merely associated with increased risks of similar cancers. Mutations in CHEK2 have been found in other hereditary and nonhereditary (sporadic) cancers. The full range of cancers associated with CHEK2 mutations has not been determined; however, studies suggest increased risks for prostate, kidney, lung, colon, thyroid, and ovarian cancers. These mutations have also been found in some brain

tumors and n osteosarcomas. Women with the CHEK2*1100delC germline mutation have a twofold increased risk of developing breast cancer compared to women in the general population (Meijers-Heijboer et al. 2002; Walsh et al. 2006; Weischer et al. 2007).

### 5.4.5 Ataxia-Telangiectasis

Ataxia-Telangiectasis (A-T) is one of the few hereditary cancer syndromes inherited in an autosomal recessive manner. This rare syndrome, affecting between 1 in 30,000 and 1 in 100,000 individuals, is characterized by cerebellar ataxia, immune defects, telangiectasias, radiosensitivity and predisposition to malignancies, especially leukemias and lymphomas (Izatt et al. 1999; Schneider 2001). While the syndrome itself places the affected individuals at a substantial increased risk of dying from cancer, the carriers of the gene for A-T are also at an increased risk of breast cancer.

The gene for A-T, ATM, is located on chromosome 11q22.3. The penetrance of this gene is 100%. The ATM gene is found in every organ and is believed to be involved in maintaining genomic stability (Savitsky et al. 1995). The increased risk of breast cancer in carriers of an ATM mutation is not well established. Earlier data indicated that a carrier female could have a five to sevenfold increased relative risk of breast cancer (Schneider 2001). Breast cancer caused by mutations in the ATM gene account for approximately 8% of all breast cancer cases (Swift et al. 1991).

### 5.4.6 Hereditary Nonpolyposis Colorectal Cancer

Hereditary forms of colorectal cancer (CRC) account for between 10 and 20% of the total number of colorectal cancer. The most common form of inherited colorectal cancer is hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome. HNPCC has an interesting history, dating back to 1895. Aldred Warthin, a well-known pathologist, published the first report on HNPCC documenting the kindred of his seamstress who died of endometrial cancer at a young age. Before genetic susceptibility testing was available, Warthin's seamstress predicted her early demise based on the fact her relatives all died of colorectal, gastric or endometrial cancer (Lynch and Lynch 2000). Many years later, international recognition of other similar families led to the official recognition of hereditary nonpolyposis colorectal cancer (Lynch and Lynch 1998).

The incidence of HNPCC is 1 in 200 to 1 in 1,000 and accounts for approximately 1–6% of colon cancers (Hampel and Peltomaki 2000). HNPCC is inherited in an autosomal dominant pattern. The penetrance of the gene is as high as 90% (Lynch and Lynch 1998).

The genes implicated in HNPCC are numerous and complex. The HNPCC genes are DNA repair genes involved in mismatch repair. As a result of mutations in the MMR genes, MSI is a characteristic of HNPCC tumors. Ninety-five percent of the tumors in individuals

with HNPCC exhibit MSI (Hampel and Peltomaki 2000). There are four genes responsible for the mismatch repair pathway associated with HNPCC (Wooster et al. 1995). The first gene, MLH1, is located on chromosome 3p21.3. The second gene, MSH2, is located on 2p22-p21. These two genes represent the majority of families with an identifiable mutation (Peltomaki and Vasen 1997). The third gene, MSH6, is located on 2p16 and the fourth gene, PMS2, on 7p22. MLH1 and MSH2 account for 90% of detectable mutations in families with HNPCC (Kohlmann and Gruber 2006).

Individuals with HNPCC have up to an 80% lifetime risk of colorectal cancer. Colorectal cancer typically occurs at a younger age with mean age of 44 years, and there is a 60–70% likelihood for tumors to be located in the proximal colon. It has also been associated with an increased risk of synchronous or metachronous colon cancer (Hampel and Peltomaki 2000). The colonic adenomas in HNPCC occur with the same frequency and in the same location within the colon as in the general population; however, the adenomas are larger, occur earlier in life, and have a higher grade of dysplasia and villous features. The characteristics of carcinomas of the colon include poor differentiation, tumor infiltrating lymphocytes, mucin and signet ring histology (Lynch and Lynch 2000).

While the average age of cancer diagnosis is 44 years, patients with HNPCC are reported to have a better survival than age and stage matched sporadic patients (Lynch and Lynch 1998). Studies have suggested this survival difference can be explained by the response to chemotherapy in HNPCC as compared to sporadic tumors (Watanabe et al. 2001). Gender differences are a possible factor in HNPCC tumor expression; the male risk of colon cancer is reported to be 91% and female risk is 69% (Dunlop et al. 1997). Women affected with HNPCC have up to a 60% lifetime risk to be affected with endometrial cancer, with the average age at diagnosis of 46 years (Aarnio et al. 1999). Improved survival in women affected with HNPCC type endometrial cancer versus sporadic occurrence is also observed (Solomon and Burt 2004).

Stomach or gastric cancers were described historically, but perhaps because of changes in Western diets and gene modifying events, the incidence of stomach cancer in HNPCC has declined. The risk of stomach cancer is reported to be as high as 13% with the average age at diagnosis being 56 years. Women are also at 12% increased risk of ovarian cancer, with the mean age at onset of 42.5 years (Solomon and Burt 2004).

The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) created the Amsterdam Criteria in an attempt to standardize the diagnostic criteria for families suspected of having HNPCC. Subsequently, Amsterdam Criteria II was proposed by the ICG-HNPCC to include additional colon cancers (Thull and Vogel 2004). In 1996, the National Cancer Institute developed the Bethesda Guidelines that described criteria for identifying colorectal tumors that should be tested for MSI. These guidelines were revised in 2002 in an attempt to increase sensitivity in identifying HNPCC MSI tumors (Umar et al. 2004). The updated Bethesda guidelines are outlined below under genetic testing for HNPCC. It is important to realize that families who do not meet Amsterdam Criteria I or II are not to be excluded from a diagnosis of HNPCC.

### 5.4.7 Familial Adenomatous Polyposis

Although familial adenomatous polyposis (FAP) is responsible for only about 1% of all hereditary colorectal cancers, it has played a large role in understanding the multiple gene model of cancer progression. The incidence of FAP is 1 in 6,000 to 1 in 13,000 individuals. Similar to HNPCC, FAP is inherited in an autosomal dominant manner with 75–80% of individuals reporting an affected parent. The remaining one-fourth to one-third of cases result from *de novo* mutations (Amos et al. 2007). The penetrance of the gene is close to 100% (Schneider 2001). The gene, located on chromosome 5q21 is called adenomatous polyposis coli protein (APC). The APC gene is a tumor suppressor and works to maintain apoptosis and decrease cell proliferation in addition to participating in many other important cellular functions. As with HNPCC, BRCA1 and BRCA2 genes, mutations in the APC gene result in truncated protein products.

Unlike HNPCC, FAP is characterized by hundreds to thousands of precancerous colon polyps that begin to develop at a mean age of 16 years (range of 7–36 years) (Solomon and Burt 2004). By age 35, 95% of individuals with FAP have colorectal polyps. Colon cancer risk is 100% by the mean age of 40 without intervention (Hampel and Peltomaki 2000). Clinical diagnosis of FAP is made in an individual with more than 100 colorectal adenomatous polyps or in any individual with less than 100 adenomatous polyps who had one relative affected with FAP (Solomon and Burt 2004).

Associated extracolonic cancers include polyps of the upper gastrointestinal tract, osteomas, dental anomalies, congenital hypertrophy of the retina pigment epithelium (CHRPE), congenital hypertrophy of the retinal pigment epithelium, soft tissue tumors, desmoid tumors, and brain cancer. Increased risks for papillary thyroid carcinoma in younger women and hepatoblastoma in affected children have also been documented (Hampel and Peltomaki 2000). Variability in clinical symptoms is quite extensive between affected family members. Gardner syndrome, a variant of FAP, is also associated with extracolonic features, osteomas, dental abnormalities, desmoid tumors and sebaceous cysts (Hampel and Peltomaki 2000).

### 5.4.8 Attenuated Familial Adenomatous Polyposis

Attenuated FAP (AFAP) is described as a form of FAP with fewer colorectal polyps (e.g. between 50 and 100 with average number of 30) that are found more proximally in the colon and at a later age than classic FAP (Lynch and Lynch 1998). AFAP is more likely to be confused with HNPCC than classic FAP for this reason. The colon polyps take on a polypoid shape in AFAP (Hampel and Peltomaki 2000). The average age for colon cancer in individuals with AFAP is 50–55 years (Solomon and Burt 2004). Multiple extracolonic polyps, such as fundus gland polyps and duodenal polyps, are detected but individuals with AFAP do not typically exhibit CHRPE characteristics. AFAP is inherited autosomally dominant and the mutations for AFAP are also located on the APC gene. Mutations are characteristically located on either the extreme 5  region of the first four exons or the 3  region of the APC gene (Hampel and Peltomaki 2000).

*MYH Associated Polyposis (MYH)* is a newly described autosomal recessive hereditary cancer syndrome associated with multiple adenomas and a phenotype similar to AFAP. The gene is located on chromosome 1 in the region 1p34.3-p32.1. A recent study by Jenkins and colleagues in 2006 found that monoallelic carriers had a threefold increased risk for colorectal cancer whereas biallelic carriers had a 50-fold increased risk for colorectal cancer.

### 5.4.9 Peutz–Jeghers Syndrome

Another rare colorectal cancer syndrome characterized by polyposis is Peutz–Jeghers Syndrome (PJS). This syndrome occurs in one of every 120,000 and has distinct clinical features. PJS exhibits an autosomal dominant inheritance pattern with the gene, STK11, located on 19p13.3 (Hemminki et al. 1998). The gene product of the PJS gene is involved in a signaling pathway for cellular apoptosis (Amos et al. 2007). In a study of 56 individuals with a clinical diagnosis of PJS in which a combination of sequence analysis and multiple ligand-dependant probe assay (MLPA) was used, the STK11 mutation detection rate was 94% (Aretz et al. 2005).

PJS is clinically diagnosed in an individual with pathognomonic hyperpigmented macules on the lips and buccal mucosa. These macules also occur on the eyes, genitalia, anus, hands and feet (Hampel and Peltomaki 2000). Individuals affected with PJS are at increased risk of multiple hamartomous polyps in the small bowel, stomach, colon and rectum, causing intususception and obstruction (Hemminki 1999). Individuals affected are 10–18 times more likely to be diagnosed with intestinal and other cancers during their lifetime. Breast and cervical cancers are also described in individuals with PJS (Boardman et al. 1998).

### 5.4.10 Hereditary Diffuse Gastric Cancer

Hereditary Diffuse Gastric Cancer (HDGC) is an autosomal dominant cancer syndrome associated with increased risks for diffuse gastric cancer and lobular breast cancer due to mutations in the CDH1 gene. CDH1 is located on chromosome 16 in the region 16q22.1. The estimated lifetime risk (by age 80) for gastric cancer for men is 67% and for women is 83%. Women also have a 39% risk of lobular breast cancer (Brooks-Wilson et al. 2004).

## 5.5 Genetic Counseling

Given the complexities involved in genetic testing and the clinical diagnosis of hereditary cancer syndromes described above, the clinician must be prepared to understand basic Mendellian inheritance as it is applied to hereditary cancer in the family, explain the hereditary components of a cancer syndrome, and understand the complexities of genetic testing.

An increase in the number of syndromes identifiable through mutational analysis coupled with the complexities inherent in testing and the potential psychological impact on affected individuals has created the need for specialized cancer genetics and high-risk clinics.

In order to accurately address patient concerns related to cancer risk, the American Society of Clinical Oncology (ASCO 2003), the American Society of Human Genetics (ASHG 1994), and the American College of Obstetricians and Gynecologists (ACOG 1997) have issued the recommendation of pre- and post-testing counseling by appropriately trained individuals with knowledge of the complex genetic issues related to hereditary cancer syndromes (ACOG 1997).

In order to meet this recommendation, genetic counseling is now offered in cancer centers and other institutions throughout the country. Genetic counseling is defined as a communication process that deals with the human problems associated with the occurrence, or risk of occurrence, of a genetic disorder in a family (ASHG 1975). Genetic counseling is offered by individuals with a M.D., Ph.D, or M.S. degree who are certified by either the American Board of Medical Genetics or the American Board of Genetic Counseling (Peters and Stopfer 1996). Genetic counselors trained at the Master's degree level attend an accredited genetic counseling training program. A national list of genetic counselors is available through the National Society of Genetic Counselors website (NSGC 2004).

The compilation of a comprehensive family history is the first step in identifying possible hereditary cancer families. As Aldred Warthin discovered upon learning about the extensive family cancer history of his seamstress, paying attention to what has happened in a family can serve to benefit future generations. A pedigree analysis is perhaps the most critical tool for defining a high-risk candidate and the need for further evaluation in a cancer genetics or high-risk clinic. The family history of cancer is often the strongest epidemiological risk factor that can be identified (Vogelstein and Kinzler 2002). Once completed, a pedigree analysis includes identifying patterns of clinical clues, based on the phenotypic expression of cancer, and matching these clues with a hereditary cancer syndrome diagnosis. Documentation of cancers, when possible, through medical records and pathology reports are necessary for the most accurate risk assessment and identification of a potential hereditary cancer syndrome.

Given the time consuming nature of taking such a thorough family history, most individuals can be screened with a family history questionnaire that can be completed by a patient either during or prior to their visit with the clinician. At the very minimum, a screening questionnaire should ask the patient to report all first and second degree relatives, the types of cancers they were affected with, ages at diagnosis, current ages or ages relatives became deceased, and should include both the maternal and paternal family histories. Other useful screening questions include the patient's ethnicity, primary cancers versus metastatic cancers and the presence of colon polyps in family histories of colon cancer.

### 5.5.1 Cancer Risk Assessment Models

After a family history has been evaluated thoroughly, the patient may be provided with a risk assessment for either being affected with cancer or if they have already been diagnosed

with a cancer, for carrying a germ line mutation for a hereditary cancer syndrome. This discussion will focus on the cancer risk assessment models that may be utilized during this portion of the evaluation.

### 5.5.2 Epidemiologic Models of Breast Cancer Risk

There are two well known models used to predict breast cancer risk in women: the Gail model and the Claus model. The Gail model is the only statistically validated model.

The Gail model was developed using risk factors for breast cancer identified in the Breast Cancer Detection Demonstration Project (Baker 1982). The model asks women to report age at menarche, age at first live birth, number of previous breast biopsies, number of first degree relatives with breast cancer, and current age. Any atypical hyperplasia diagnosed from biopsies is also evaluated at this time. Using this data, the Gail model provides women with both 5-year and lifetime risk of developing breast cancer. An updated version of the Gail model can be downloaded from the National Cancer Institute website (NCI 2007). The updated version provides risks for invasive breast cancer only, derives baseline incidence rates from SEER data, and includes a separate baseline incidence for black women (NCI 1998).

The Gail model was developed prior to the discovery of the BRCA1 and BRCA2 genes and therefore only limited family history is included. This leads to underestimation of cancer risks in women with a more extensive family history of early-onset breast cancer, ovarian cancer, male breast cancer or paternal relatives with breast cancer. Therefore, the Gail model is not recommended for use in high-risk families (Barnes-Kedar and Plon 2002).

The Claus model consists of published tables to estimate risk of breast cancer over time. This model requires family history information, including paternal history and occurrences of ovarian cancer to calculate cancer risk (Domchek et al. 2003). Individuals receiving risk values from this model are encouraged to remember that the model can be imprecise since it does not account for subtle features of hereditary cancer. Like the Gail model, Claus should also be avoided in individuals with a strong family history of cancer. New models are under development that will incorporate both family history and individual risk factors (Tyrer et al. 2004).

### 5.5.3 Genetic Testing Models

The patient is offered an estimation of the prior probability of an individual to carry a gene mutation in one of the hereditary cancer susceptibility genes. Prior probabilities models are available for hereditary breast and ovarian cancer and for HNPCC.

Models for predicting breast cancer mutation probability include the Couch, Shattuck-Eidens, Frank and the Berry–Parmigiani–Aguilar or BRCAPRO models (Claus et al. 1994; Shattuck-Eidens et al. 1997; Frank et al. 1998; Parmigiani et al. 1998). In addition, a computer program has been developed (CAGene) that calculates risk by incorporating

the Couch, Shattuck-Eidens and BRCAPRO models. It also provides mutation prevalence estimates from Myriad Genetic Laboratories. This program is offered as a free service on the internet (Parmigiani and Wang 2004). For a more extensive review of each of these models, see Domchek et al. (Domchek et al. 2003).

There is a colorectal cancer model available (the Wijnen model) to predict an individual's probability for testing positive for a gene mutation for HNPCC. The Wijnen model predicts prior probability for a mutation in either the MLH1 or the MSH2 genes. The average age of diagnosis of colorectal cancer, presence of endometrial cancer in the family, and the Amsterdam criteria are entered into the program to calculate prior probability risk. If an individual's prior probability for carrying a mutation on one of these two genes is calculated to be equal to or greater than 20%, Wijnen suggests clinicians first offer germ line testing without MSI testing (Wijnen et al. 1998). CRCAPRO is a statistical model that assesses the probability that an individual carries a germline deleterious mutation of the MLH1, MSH2, and/or MSH6 genes. It is based on personal and family history of colon and endometrial cancer and uses a Mendelian approach assuming autosomal dominant inheritance. Age dependent penetrance and prevalence data are based on review of the literature. It is available as part of the CaGene program by David M. Euhus mentioned above.

### 5.5.4 Informed Consent Prior to Genetic Testing

The availability of the models described above to predict prior probability of a germ-line mutation will likely result in more individuals and families requesting genetic testing. The issues surrounding genetic testing for hereditary cancer syndromes have been debated extensively. The conclusion of these debates is that the decision to undergo genetic testing should remain a personal choice. Therefore, proper informed consent requires a thorough discussion with the patient prior to genetic testing. A comprehensive informed consent has been documented and includes a discussion of, but is not limited to: the purpose of the test (including testing which is part of a research protocol, costs, turnaround time, and documentation of results), the predictive value of a positive, negative or indeterminate result, and corresponding cancer risk information. It should also include options for cancer risk management if the test is positive, negative or indeterminate, the possible psychological implications of testing, individualized assessment of insurance, employment discrimination risks, and alternatives to genetic testing, such as the possible delay of decision making to a future date (Geller et al. 1997).

The importance of adhering to this informed consent process was demonstrated in a study that examined the use and interpretation of genetic testing for mutations on the APC gene. The study found that 20% of physicians ordered testing erroneously for FAP, only 18.6% of individuals in this study received genetic counseling before the test and in 31.6% of the cases the physicians misinterpreted the results (Giardiello et al. 1997).

In direct response to the complexity of genetic testing issues, as well as in an attempt to define those who would most benefit from genetic testing, the American Society of Clinical Oncology (ASCO) recommends genetic testing be offered when personal or family history is representative of a possible hereditary cancer syndrome, the test is able to be adequately interpreted, and the results from the genetic test will assist with medical management decision for the individual and their family. ASCO guidelines go on to state

that clinicians offering genetic testing must include pre- and post-test genetic counseling, documentation of a family history of cancer, and must provide a risk assessment as well as discuss options for prevention and early detection (ASCO 2003).

## 5.6 Genetic Testing

### 5.6.1 Genetic Testing for Hereditary Breast and Ovarian Cancer

Although there is no recommended numerical "prior probability" to indicate when to offer gene testing for the BRCA1 and BRCA2 genes, responsibility of when to offer genetic testing is based on the judgment of the clinician. Once an individual has undergone a family history evaluation, provided with risk assessments, demonstrated to meet the criteria outlined by ASCO guidelines, genetic testing is typically offered. Eighty to ninety percent of the time, hereditary breast and ovarian cancer is caused by BRCA1 and BRCA2 mutations. A recent study from Sweden reported one in ten ovarian cancer patients carry a BRCA1 or BRCA2 mutation (Malander et al. 2004). Genetic testing for the BRCA1 and BRCA2 genes is one of the most requested genetic tests for hereditary cancer.

The standard protocol for initiating genetic testing is to test for the BRCA1 and BRCA2 genes on the affected individual in a family before offering testing to the at-risk relatives. Given the high incidence of "private mutations," and the likelihood of other hereditary breast cancer genes yet to be discovered, the results will be most definitive in an individual already affected with cancer. If no living affected relatives are available, it is feasible to begin testing close family members.

Genetic analysis of the BRCA1 and BRCA2 genes include complete sequencing with results available in three to four weeks. In 2007, the price of this test was approximately $3,120 (US dollars). Most insurance companies will cover at least a portion of the cost of this test. Individuals of Ashkenazi Jewish descent can elect to undergo the ethnicity panel, screening for the three common founder mutations. The cost of this test in the US was approximately $460 in 2007. Issues related to health insurance discrimination are becoming less common. Most states currently have legislation to prohibit health insurers from using the results from a genetic test to deny coverage, set premiums or drop coverage. On May 21, 2008, US President George Bush signed the Genetic Information Nondiscrimination Act (GINA) into law. GINA prohibits the use of an individual's genetic information in determining eligibility, premium or contribution amounts by group or individual health insurers. GINA also prohibits the use of an individual's genetic information by employers.

A positive result from a BRCA1 or BRCA2 genetic test identifies a deleterious mutation in the individual tested. Relatives of the BRCA positive individual can then be offered testing for the same mutation, which is called a single-site analysis. The siblings and offspring of a carrier proband are at a 50% risk to inherit the same mutation. In 2007, the cost for the single-site analysis was $385 in US dollars. In some families, more than one deleterious mutation is identified, complicating the testing protocol. In these situations, it is recommended that all at risk relatives undergo genetic counseling prior to undergoing genetic testing.

A negative result indicates no deleterious mutations were identified in the examined gene. Negative results are considered either a true negative or uninformative. A true negative occurs when a mutation has already been identified in an affected family member. Therefore, the individual who tested negative did not inherit the mutation in their family history. A recent study by Smith and colleagues (Smith et al. 2006), found that in these identified high risk families, even women who test negative for the familial BRCA1/BRCA2 mutation have a two to threefold increased lifetime risk (by age 70) of breast cancer consistent with genetic modifiers.

A result is uninformative when an affected individual tests negative, yet their family history is indicative of the breast and ovarian syndrome. In families in which a true autosomal dominant cancer syndrome is present, but no mutation has been located, either the family carries a different susceptibility gene or there is a mutation on the BRCA1 or BRCA2 gene that is not detectable by current testing methods. In families for which no mutation is identified, yet there is a pedigree suggestive of hereditary cancer, risk assessment for cancer must be based on the family history.

A negative test result in an individual not affected with cancer may also be uninformative when they are tested before an affected relative. Until the affected relative is tested, it is impossible to know whether the results were negative because they did not inherit the cancer susceptibility gene or because there is no such mutation in the family. If affected relatives are unavailable to clarify the results, risk assessment should again be based on the family history of cancer.

The final possible result for individuals undergoing BRCA1 and BRCA2 analysis is a variant of uncertain significance. Variants occur in 10% of the samples that are analyzed at Myriad Genetics, Inc. (Salt Lake City, UT) (Frank et al. 1998). This result is most commonly reported when a missense mutation is identified. Missense mutations may or may not affect the protein function of the gene product.

Until a protein assay is developed to determine the effect on protein expression, interpretation of this result is based on clinical observation. All affected relatives in a family are offered testing to determine if the variant tracks with cancer. Receiving a result of a variant of unknown significance leaves the clinician with limited information about cancer risks. Medical management decisions cannot be made from this result.

### 5.6.2 Genetic Testing for Hereditary Nonpolyposis Colon Cancer

Given that 95% of tumors in individuals with HNPCC are MSI positive, testing the tumor for MSI is recommended prior to genetic testing (Hampel and Peltomaki 2000). The Bethesda guidelines have been developed to identify individuals affected with colon cancer who would be appropriate candidates for MSI testing. The revised Bethesda Guidelines recommend testing tumors for MSI in individuals who meet one or more of the following criteria:

1. Colorectal cancer diagnosed before age 50
2. Presence of synchronous, metachronous colorectal or the HNPCC associated tumor irrespective of age at diagnosis

3. Colorectal cancer with microsatellite instability and the presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation or medullary growth pattern
4. Colorectal cancer diagnosed in one or more first degree relatives with an HNPCC-related tumor, and one cancer diagnosed less than 50 years old
5. Colorectal cancer diagnosed in two or more first or second degree relatives with HNPCC-related tumors, irrespective of age at diagnosis (Umar et al. 2004)

If the tumor is MSI positive, germline testing is offered. The probability of detecting a germline mutation when an individual meets Amsterdam Criteria I is 40–80%. When the Bethesda Guidelines are met and a tumor is MSI positive, the probability of detecting a mutation nears 50% (Kohlman and Gruber 2006). Since 5% of tumors from verified HNPCC cases do not exhibit MSI, screening negative for this feature does not rule out the diagnosis of HNPCC. In addition, many individuals with colon cancer have MSI but do not have HNPCC. Currently, clinical testing for mutations in the MLH1, MSH2, and MSH6 genes is available.

### 5.6.3 Genetic Testing for Familial Adenomatous Polyposis

Germline testing in individuals with a clinical diagnosis of FAP is typically preformed to identify a mutation in the family so that at-risk relatives can undergo genetic testing. As with hereditary breast and ovarian cancer syndromes and testing for the BRCA1 and BRCA2 genes, it is emphasized in testing individuals for the APC gene mutations, that a clinical diagnosed individual be tested prior to the at-risk relatives. Molecular testing is also offered to confirm the clinical diagnosis in patients with FAP who may have less than 100 adenomatous polyps. Full gene sequencing will detect up to 90% of mutations on the APC gene. A protein truncation test, when performed alone will be positive in about 80% of individuals affected with FAP. If there are at least two affected individuals available for testing, linkage analysis can also be attempted and is informative in 95% of families tested (Solomon and Burt 2004).

Because screening for FAP begins as young as 10 years old and children who carry an APC gene mutation are at risk for hepatoblastoma, molecular genetic testing is offered to at-risk children under the age of eight. While there is no evidence of psychological problems when testing is performed this early, some recommend that long-term psychological counseling be provided to these individuals (Solomon and Burt 2004).

Genotype-phenotype correlations are predicted to be available in the future allowing for individualized preventative screening and medical management recommendations.

### 5.6.4 Genetic Testing for Attenuated FAP

Because the APC gene is responsible for both FAP and AFAP, the recommendation for genetic testing is the same. The mutations for attenuated FAP are located on the far ends,

the 3′ or 5′ ends of the gene. Since AFAP and HNPCC can present in a similar manner, molecular testing may be used to confirm or rule out a diagnosis. Molecular testing is offered to individuals 18 and older for AFAP given the later age of onset of symptoms (Solomon and Burt 2004). Genetic testing is also available for MYH-associated polyposis with both common mutations and founder mutations already described.

## 5.7 Cancer Screening, Surveillance and Prophylactic Management for Hereditary Cancer Syndromes

The purpose of offering a risk assessment evaluation and genetic testing is to identify individuals at increased risks for cancer prior to cancer initiation so that screening and prevention strategies can be implemented. Medical management guidelines have already been published for hereditary breast and ovarian cancer as well as hereditary colorectal cancer syndromes. The major components of these guidelines will be described.

### 5.7.1 Prevention Strategies for Hereditary Breast/Ovarian Cancer

The manner in which individuals incorporate positive genetic test results for a BRCA1 or BRCA2 mutation is the initial step in understanding the effect genetic testing has on screening and surveillance and other prophylactic options. Behavioral modification, when necessary, is the first line of defense towards this effort. Currently several studies are working to identify the long-term effects of learning gen etic predisposition to cancer prior to a cancer diagnosis (Risch et al. 2001). Early results indicate that fewer women than expected opted for prophylactic surgery after testing positive for a BRCA1 or BRCA2 gene (Lerman et al. 2000). More research is necessary to understand the psychological effects of identifying a positive mutation. The findings of these studies will aid in developing behavioral interventions to increase understanding of, and adherence to, available options for this at-risk population.

The options available to women who are at an increased risk of breast and ovarian cancer can be divided into three categories: screening and surveillance; prophylactic surgery; and chemoprevention. Screening recommendations for women positive for a BRCA1 or BRCA2 mutation were written by a NIH consensus panel and published in 1997 (Burke et al. 1997) and are summarized below.

- *Breast cancer screening*: Clinical breast exam every 6 months, mammograms every 6–12 months beginning at age 25, and education regarding monthly self-examination.
- *Ovarian cancer screening*: Annual transvaginal ultrasounds, serum CA-125 levels and biannual pelvic exams. This information is based on expert opinion with no data indicating that these screening methods will reduce mortality from ovarian cancer in women who test positive for a BRCA1 or BRCA2 mutations (Madalinska et al. 2007; Risch et al. 2001)

› *Colon cancer screening*: Male and female carriers of the BRCA1 and BRCA2 are informed of the possible increased risk of colorectal cancer and advised to follow the guidelines for screening published for the general population (Liede et al. 2004).

The recommendations above have not been studied to prove benefit of surveillance on cancer-related mortality in women who are BRCA1 or BRCA2 mutation carriers. These recommendations are therefore based on expert opinion only. New studies have recently been published indicating an increased sensitivity of detection of breast cancer by magnetic resonance imaging (MRI) compared to standard mammography (Kriege et al. 2004; Warner et al. 2004). While MRI has shown to be more sensitive than mammography, the specificity is lower for MRI versus mammography (Kriege et al. 2004; Warner et al. 2004). In addition, these studies still have not proven benefit of MRI surveillance on cancer-related mortality in women who carry a BRCA1 or BRCA2 mutation.

Male carriers of mutations in BRCA1 or BRCA2 should also be offered appropriate screening and surveillance. Currently there is no standard recommendation for breast cancer screening in male carriers. Men can be advised to perform breast self-examination and contact their physician if any changes are detected. Screening with mammography is not typically recommended for males. Prostate cancer screening includes an annual prostate specific antigen (PSA) test and a digital rectal examination for men over the age of 40 (Liede et al. 2004).

### 5.7.2 Prevention Strategies for Hereditary Colorectal Cancer Syndromes

Two groups have published screening guidelines for individuals with HNPCC. The ICG-HNPCC first published their guidelines in 1996, which were reviewed by a task force from The Cancer Genetics Studies Consortium in 1997 (Weber 1996; Burke et al. 1997). In general, both groups recommended colonoscopy every 1–3 years, starting at age 20–25, for all at-risk relatives.

Initial recommendations for endometrial cancer screening were changed by the task force in 1997. Currently, screening for endometrial cancer is recommended beginning from age 25–35. Screening methods include either annual endometrial aspirate (in premenopausal women) or transvaginal ultrasound with biopsies of suspicious areas. Screening for gastric cancers or urinary tract cancers are only recommended if these cancers occurred in other family members. Due to lack of sufficient data proving efficacy, serum CA-125 screening for ovarian cancer has not been recommended. Several studies have reported on the efficacy of such screening strategies in HNPCC families and conclude a decrease in incidence and mortality from colorectal cancer (Vasen et al. 1998).

Guidelines for individuals with FAP have been updated and published by the Mayo Clinic. Screening for FAP begins as early as age ten, with prophylactic colectomy recommended between 17 and 20 years of age. An individual who does not undergo a proctocolectomy will require surveillance of the rectal stump every 6 months. Individuals with FAP are also encouraged to undergo baseline endoscopic screening for adenomas in the stomach and duodenum and a follow-up every 3–5 years after colectomy (Hampel and

Peltomaki 2000). Surveillance for colon cancer in individuals with AFAP is similar, but typically begins at age 20 with annual colonoscopy.

To screen for other cancers, sonography and palpation is recommended in young women with FAP. Liver palpation, serum alpha-fetoprotein measurement and ultrasound are recommended for at-risk children until age six (Hampel and Peltomaki 2000).

### 5.7.3 Prophylactic Surgery

Early studies have indicated a 90% risk reduction in breast cancer incidence and mortality after prophylactic mastectomy in high-risk women (Hartmann et al. 1999). A recent study reported a 95% reduction in breast cancer in BRCA1 and BRCA2 mutation carriers after prophylactic mastectomy with prior or concurrent bilateral prophylactic oophorectomy (Rebbeck et al. 2004). Bilateral prophylactic oophorectomy (BPO) is offered for women at increased risks for ovarian and breast cancer. Rebbeck and colleagues studied effects of BPO in carriers of a BRCA1 mutation and reported a 50% reduction in risk of breast cancer (Rebbeck et al. 2004). Early studies have suggested increased life expectancy in BRCA1 and BRCA2 carriers who undergo prophylactic mastectomy and oophorectomy (Schrag et al. 1997).

Prophylactic surgery has not been proven to be effective in individuals affected with HNPCC, but the option for either subtotal colectomy (or proctocolectomy) with ileorectal anastomosis should be given to the patient after the first colon cancer diagnosis is made or when adenomas are diagnosed. In addition, women should be offered total abdominal hysterectomy and bilateral salphingooophorectomy (King et al. 2000).

### 5.7.4 Chemoprevention

In the primary prevention setting, tamoxifen in high risk women has reduced breast cancer incidence by approximately 50% (Fisher et al. 2005). As a result, tamoxifen became the first drug to be approved by the FDA for use as a preventive agent against cancer in women at very high risk. Subgroup analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 trial found that tamoxifen reduced breast cancer incidence by 62% in BRCA2 carriers but failed to do so in BRCA1 carriers when started at age 35 or older (King et al. 2001). Prophylactic use of tamoxifen is also beneficial for CHEK2 mutation carriers, who are more likely to develop steroid-receptor-positie tumors (Schmidt et al. 2007). Tamoxifen chemoprevention is reserved for women at high risk because of the associated adverse effects such as endometrial cancer and thromboembolic events (Fisher et al. 2005). A second prevention trial conducted by the NSABP (P-2) compared tamoxifen with raloxifene and showed that raloxifene was equivalent to tamoxifen in the prevention of invasive breast cancer (Vogel et al. 2006). Investigators are also exploring the possible prevention effects of oral contraceptive use in women who carry a BRCA1 or BRCA2 mutation. Initial studies have suggested a reduction in the risk of ovarian cancer in BRCA1

or BRCA2 carriers after oral contraceptive use for an average of 4 years (Narod et al. 1998). Several chemoprevention trials are underway to test sulindac and other nonsteroidal anti-inflammatory drugs to prevent the development and advancement of polyps in FAP individuals (Hampel and Peltomaki 2000).

## 5.8 Conclusion

Cancer genetics and hereditary cancer syndromes are opening up an entirely new arena for cancer prevention. Education is the first step in the process of cancer prevention for hereditary cancer syndromes.

Compiling a detailed three generation family history is perhaps the single most important preventative action clinicians can do for their patients. Understanding the concepts of cancer genetics and inheritance of hereditary cancer allows the clinician to properly identify those at increased risk.

Educating the general population on the importance of maintaining accurate family records, especially of diagnosis and age of onset is also necessary. Educating individuals and families and helping them appreciate the role of heredity in cancer and will empower patients to learn more about their family history.

Understanding the genetic risks conferred to patients who carry hereditary cancer gene mutations allows for more personalized medical management strategies. Information provided through the genetic test results may serve to increase an individual's lifespan and to prevent cancer occurrence.

Our continued understanding of the molecular genetics of cancer at the cellular level is leading to better targeted therapies and chemoprevention options. Learning how the environment can affect a gene's function will allow individuals to modify lifestyle choices and play a part in their own cancer prevention.

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# Human Categories and Health: The Power of the Concept of Ethnicity

6

K. Coe, C.T. Palmer

## 6.1 Introduction

The inclusion of racial and ethnic minorities in cancer prevention studies is a scientific, logistic, cultural, and ethical issue. A great deal has yet to be learned about cancer in these populations: Why are some racial and ethnic minorities more likely to get certain cancers, be diagnosed at later stages, and die of their disease? If it is true that they respond differently to medications and treatment modalities, why does that occur? Why does their participation in many clinical studies remain so low? For a number of reasons, the inclusion of racial and ethnic minorities in cancer research trials is an important concern for researchers. There are a growing number of reports of robust relationships between race/ethnicity and health outcomes (Kato 1996; Patrinos 2004).

The answer to these questions, we argue, lies in a deeper understanding of the meaning of race and ethnicity. Definitions of both terms – race and ethnicity – are inconsistent and unclear; researchers employ the concept to measure every important indicator associated with inequality or difference. Socioeconomic status, cultural lifestyles and values, and genetic predispositions are all being measured by these variables. A discussion of the multifactorial elements, implicit or explicit, in the use of the terms race, ethnicity, and culture can be used to point out the elements that are fundamental to these terms and that may influence health behaviors and outcomes.

We will argue that the concept of ethnicity is the more useful term for understanding health than are concepts such as race, or even concepts of culture, because it has, since the origins of the term, focused on the role of traditions while acknowledging that genetics are involved. Ethnicity is best understood when we adopt the interactionist view that specifies that both genes and environmental factors interact in human development and play a role in health and illness. By taking an interactionist view, more successful cancer prevention interventions and research trials might be developed.

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## 6.2 Background

Certain cancers are more common in ethnic minorities than they are in other populations. Even among those populations in which cancer incidence rates are lower, the mortality rates may be higher. Today, Hispanic/Latino women who live in the United States, when compared to non-Hispanic white women, have twice the incidence rate and 1.4 times the mortality from cervical cancer (Reynolds 2004). Relative to non-Hispanic whites, a greater proportion of African American, American Indian, Hawaiian, and Hispanic patients are diagnosed with testis cancer at later stages (Lou Biggs and Schwartz 2004). American Indian women, when compared to their white counterparts, have a significantly lower 5-year cancer survival rate (Samet et al. 1987).

In addition, the cancer rates in racial/ethnic populations are rising; during the last century, cancer was a disease so seldom diagnosed among American Indians that anthropologists were led to argue that they were immune to cancer or had some natural protection from the disease (Hrdlicka 1905; Leven 1910). Among the First Nations people of Canada, cancer incidence rates increased significantly between 1968 and 1975 and from 1984 to 1991 for all cancers and for the major cancers (e.g., breast, lung, prostate and colorectal) (Marrett and Chaudhry 2003). Further, there are regional differences in the types of cancers diagnosed. For example, American Indians in New Mexico are at higher risk of gestational trophoblastic neoplasia (Smith et al. 2004).

Despite this evidence of increasing cancer mortality and morbidity, cancer screening rates are lower in ethnic minority populations than in whites (Walsh et al. 2004). Substantial subgroups of American women, specifically those of racial/ethnic minorities, have not been screened for cervical cancer nor are they screened at regular intervals. Hispanic women are less likely to receive screening mammograms than are white or African American women (Bazargan et al. 2004), even though breast cancer is the leading cause of cancer-related deaths in the Hispanic population (Darling et al. 2004). There are also disparities related to treatment; in a recent study of prostate cancer, racial/ethnic minorities were found to be less likely to receive definitive therapy for the treatment of disease (Underwood et al. 2004).

The factors contributing to these disparities, to the increased likelihood of racial/ethnic minorities to get certain cancers, to die of certain cancers, to be diagnosed at a late stage in the disease progression, and the decreased likelihood to be screened for cancer or to receive the best treatment, remain elusive. One problem with research in ethnicity and health is that researchers employ the term to measure every important indicator associated with inequality or difference. The independent importance of this variable, however, is evident in the fact that even if we control for other factors, race and ethnicity are predictors of disparities (Palacio et al. 2002; Cohen 2003; Opolka et al. 2003; Guller et al. 2004).

To begin to investigate these issues, it is important to understand not only what race and ethnicity are, but why ethnicity might impact health. Only then can we use this knowledge to develop an effective and fruitful approach to health disparities research (Ashing-Giwa et al. 2004). Due to the scientifically verifiable robust relationships that exist between racial/ethnic groups and health outcomes (Kato 1996; Patrinos 2004), the term “race” has largely been discarded and the term ‘ethnicity’ is increasingly being used as categorical variables in social research related to cancer and its prevention. While an association between

ethnicity and health outcomes does exist, the use of ethnicity as a grouping variable in health research is disturbing to scientists. It is poorly defined, is not objectively measured, and cannot be studied in a true experiment. Thus, scientific conclusions about the causal relationship between ethnicity and health are difficult to make (Kato 1996).

The failure of scholars to agree on the traits necessary or sufficient for ethnic membership has led to controversy, with some researchers dismissing the term as a political category that is otherwise meaningless. The strong association between ethnicity and health indicates that we should not consider abandoning the term, but rather suggests that it deserves careful study (Kato 1996). This is true not only for the study of health and ethnicity, but for the study of ethnicity in general because "the academic specialty usually called 'race and ethnic relations' is rich in literature but poor in theory " (Van den Berghe 1981).

## 6.3 Race and the Failure of Attempts to Define Ethnicity

Much of the confusion related to the term ethnicity is the result of attempts to equate ethnicity with either the biological or genetic concept of race or the environmental concept of culture. These attempts have failed because ethnicity incorporates both genetic and environmental factors.

Race originally was a taxonomic term coined by biologists to refer to a subspecies. For example, Kroeber wrote that a race "is a group united by heredity, a breed or genetic strain or subspecies" (Kroeber 1923). It corresponded, he continued, "to a breed in domestic animals" (Kroeber 1923, p. 75). Hoebel was yet more explicit. "A race is a biologically inbred group possessing a distinctive combination of physical traits that tend to breed true from generation to generation" (p. 69). The assumption that race was defined by genetic differences between races, often mislabled as biologic differences, is illustrated by the fact that such a grouping is at times called a Mendelian population (Zuckerman 1990). The genetic differences responsible for the physical and behavioral similarities (e.g., phenotypes) found among individuals in Mendelian populations occur because all individuals in this small population share relatively recent common ancestry.

It seemed obvious to early explorers that geographically isolated human populations also seemed to be distinguishable in terms of phenotypic characteristics. Among the earliest published human racial classifications was Francois Bernier's *Nouvelle division de la terre par les diffrents espces ou races qui l'habitent* ("New division of Earth by the different species or races which inhabit it") (Bernier 1684). In the nineteenth century, race continued to be identified based on similarity in appearance and was generally measured by skin color and other obvious morphologic characteristics. As Kroeber (1923) recognized, in gross physiology, all human races were much alike. However, it was also true that a great many distinct characteristics distinguished different categories of humans (e.g., skin and hair color, stature, cephalic index, nasal index, texture of the hair, hairiness of the body, prognathism). It was not clear which of these markers might be the most important in classifying races. While heredity was involved (in that children resembled their parents), these physical similarities were felt to be related to a common geographic place of origin:

the white race, with its origin in the Caucasus; the yellow race, with its origin in Mongolia; the black race, with its origin in Ethiopia or Africa (Cuvier 1848; Gould 1981). Racial categories were seen as typologies; one of such types (black) differed from another (white, yellow, or red) (Crews and Bindon 1991). Early discussions of the meaning of race focused solely on physical characteristics; culture was ignored.

By the twentieth century, the conditions once considered to be necessary or sufficient for membership in a particular human race were beginning to be problematic. Although the characteristics that determined racial membership were due to genetic similarities resulting from common ancestry and reproductive isolation, these characteristics were beginning to appear to be arbitrary. Researchers began to argue that the term race might not be an appropriate term for human groups. In fact, a long tradition of scholarly research has argued that race is an arbitrary system of visual classification that fails to and, indeed cannot, "demarcate distinct subspecies of the human population" (Fullilove 1998).

Researchers began to argue that similarity in physical and behavioral features could be a product of genetic admixture brought about by the migrations that began to occur early in human prehistory or it could also be due to convergent evolution, with similar environmental pressures selecting for similar responses, including both physical and behavioral characteristics. People in Africa and in India, while perhaps sharing a dark skin, are not closely related; their dark skin may be an adaptation to particular environmental pressures. Among humans, as among other species, physical appearance is neither a reliable nor a valid indicator of relatedness or shared descent. "We are genetically far more nuanced and variable than is reflected in just skin coloration" (Patrinos 2004).

While some of the debate was related to an objection to the concept of humans as animals, there were ample and significant reasons for rejecting the concept of race. While subspecies and Mendelian populations are usually small, races are often large. In addition, as races were identified by ideal type, intermediate types that failed to match the ideal, such as brown skin as opposed to black, led to the breakdown of the typology. These variants could neither be forced into one of the ideal types nor could these variants be ignored (Crews and Bindon 1991).

Particular mate choices, including a long history of arranged marriages and tribal endogamy, can lead to rapid selection for certain morphological characteristics. "Cultural restrictions for mate selection based on external morphological characteristics may lead to rapid differentiation of skin, eye, or hair color between populations while leaving aspects of basic biology and energy metabolism relatively unaffected" (Crews and Bindon 1991). In sum, the visual differences once seen as so important, are actually only superficial and, in fact, the racial categories that were once promoted did not typically reflect the factors, such as genomics, that are actually now seen as important for classification. Most importantly, studies of actual genetic clinical distributions have found that the vast majority of genetic differences occur within any given racial category, with only a very small percentage of the variation occurring between racial classifications (Lewontin 1972). As early as 1923, Kroeber argued that "variations between individuals of the same race are often greater than differences between the races" (p. 126). These studies led to the realization that phenotypic differences do not necessarily indicate that there are dramatic genotypic differences. In addition, there was the real fear that a clear designation of racial categories would contribute to racism. Thus, the concept of race fell into disfavor.

The rejection of the concept of race led to the popularity of ethnicity as a replacement in both scientific and nonscientific research (Bhopal and Donaldson 1998). The roots of the term *ethnic* are Greek (as well as Latin *ethnicus*, German *ethnikos* and French *ethnique*), meaning a nation or people and its cultural practices. According to *Webster's Dictionary*, by 1957, ethnicity was a term most commonly used to refer to nations or groups that were neither Christian nor Jewish, but heathen. In other words, ethnicity was used to distinguish "them" from "us," with us being the explorers and scholars writing about exotic peoples. The second meaning of ethnic used in the dictionary referred to "any of the basic divisions or groups of mankind, as distinguished by customs, characteristics, (or) language." Ethnic groups, following from this, were identifiable and distinguishable on the basis of cultural traits, which included not only shared language and other cultural practices, but also the knowledge, attitudes, and beliefs shared by members of a cultural group.

At its origin, ethnicity was used to distinguish cultural homogeneity (ethnic group) from biological homogeneity (race) (Damon 1969). The use of the term "ethnic group" was promoted, based at least partially on the idea that it would stimulate discussion as to its meaning and thus clarify the meaning of race (Montagu 1962). Attempts to replace the term race with the term ethnicity, and thus shift from genetic racial categories to cultural ethnic categories, unfortunately, have not proven to be as successful as was initially hoped (Crews and Bindon 1991).

The problem is that the term ethnicity is not simply a synonym for culture. While ethnicity has been tied to cultural behavior since its origin, there is an association with other traits that are not necessarily cultural. Although researchers often deny that there is a genetic component to ethnicity (Amick 1995), ethnicity does in fact have a genetic aspect. The realization that ethnicity is not just cultural has lead to the persistence of attempts to equate ethnicity with race. For example, the terms race and ethnicity are still often found together, and at least implicitly are often used as synonyms (Bulmer 1999; Kromkrowski 2002; Scupin 2003).

Ethnic groups are also often described as being sub-groups of races, and to the extent that ethnicity is seen as a sub-grouping of race, it has an implied genetic component (Miranda 1997). While physical appearance may be due to convergent evolution, it can be related to actual genetic differences (Crews and Bindon 1991). Looking like, or resembling, the other members of your ethnic group was important (Westermarck 1921). In fact, non-cultural phenotypes are the most reliable markers of ethnicity (Van den Berghe 1981). For example, an individual who looks Asian is classified as Asian, even if he or she follows no Asian cultural practices at all (Kato 1996). Despite attempts to define ethnicity in purely cultural terms, ethnicity is popularly seen as biological, as related to ancestry and the genes and traditions inherited from them (Nagel 1996). The failure of the term ethnicity to completely replace race indicates that there is a clear sense that something important would be lost by a complete shift from a biological or genetic to a cultural definition.

There are additional contradictions encountered when ethnicity is used interchangeably with race. It is recognized that racial categories "are not co-extensive with any existing ethnic group" (Crews and Bindon 1991). Serbians and Croatians are classified as white; however, few would argue that they represent a single ethnic group (Crews and Bindon 1991). As a result, many researchers have returned to using the term race, but have redefined it in an attempt to make it more applicable to human categories (Bhopal 1999). Some researchers

use the term race, but assert that there are no identifiable genetic differences between races that can explain disparities in health (Fullilove 1998). If no genetic differences exist, it is unclear why race should be used at all instead of simply using cultural differences for these categorizations. Despite all of the problems with the term race, it has neither been abandoned nor replaced with culture because of the view that ignoring race in health statistics could lead us to ignore the disparities these statistics bring to light (Buehler 1999).

Instead of attempting to redefine race, the best solution may be to more clearly define ethnicity. The inability to define ethnicity in exclusively genetic or cultural terms implies that both factors need to be incorporated. Ethnicity must be understood within the context of the interactive view of human development.

## 6.4 The Interactive View of Human Development

An accurate understanding of the relationships among race, ethnicity, and culture requires an understanding of the interactive theory of human development (Alcock 2001). According to the interactive theory, the development of humans and all living organisms involves an incredibly complex interaction of both genetic and environmental factors. This requires an understanding of the terms biologic, genetic, environmental, innate, learned, culture, and tradition as well as an understanding of their interactions.

### 6.4.1 Biologic, Genetic and Environmental

Much of the confusion over the meaning of ethnicity stems from the meaning of biological difference. This can be avoided by simply remembering that biology is the study of life (Thornhill 2000). Hence, to describe something as biological is simply declaring it living. Often, biological is incorrectly interchanged with the term genetic. These terms are not interchangeable; the traits of living, biological things (e.g. organs, limbs, skin, eye color, behaviors) are all phenotypes. In contrast, the genes within any individual organism are merely its genotype. Phenotypes are not the same as genotypes. Any part of an organism's phenotype is the result of an interaction between genes and environmental factors.

To understand this point it is crucial to recognize that environmental factors include more than just those things normally associated with the environment. To a biologist, the environment includes all exogenetic factors; that is, everything that the genes of an organism interact with during the development of an organism, both before and after birth. Such factors include not only the air, water, nutrients, and other chemicals the organism consumes, but the other living things, including humans, the organism encounters. Further, the environment includes not only things external to the organism, but also chemicals within the organism that interact with genes. Hence, *everything* biologic is the product of both genes and environmental factors. Even an individual cell, the most fundamental building block of any larger organism, is a product of genes and certain aspects of the environment. The constant intertwining of genetic and environmental factors continues throughout the life of the organism. This is true even for behavior, the muscular-induced movements of organisms.

### 6.4.2 Learned and Innate

Since behavior is part of the phenotype of an organism, it is a product of the interaction of both genetic and environmental factors. This fact makes the distinction between learned and innate behaviors untenable. A behavior is learned when a specific environmental factor has been identified as necessary for the behavior's occurrence. For example, when it is identified that a person must get on a bicycle and fall off several times before they are able to ride successfully, riding a bicycle is claimed to be a learned behavior. However, focusing on only this particular necessary environmental factor causes people to overlook all of the other factors that are also necessary for a human to ride a bicycle. These include all kinds of other environmental factors during the development of the child such as oxygen, water, and nutrients, as well as certain genes that enable physical functioning to interact with environmental factors. Remove any of these necessary environmental or genetic factors and bicycle riding will never occur. All behaviors that we call learned are the result of *many environmental factors and genes*.

A behavior is typically called innate when certain environmental factors are identified that are *not* necessary for a behavior to occur. For example, the sucking behavior of infants occurs before the infant ever sees another infant making the motion or is even exposed to a nipple. This is claimed to be an innate behavior. However, there are many other factors that occur during development that are necessary if an infant is to perform the sucking motion. These include not only certain genes, but the interaction of those genes with such environmental factors in utero, including maternal nutrition. All behaviors that we call innate are also the result of *many environmental factors and genes*.

Since all behavior, whether it is claimed to be learned or innate, is the result of an interaction between genes and numerous environmental factors, the distinction between learned and innate behavior has no meaning. The debate between whether a behavior is learned or innate should be replaced with attempts to identify what genes and what environmental factors are necessary to produce a given behavior or for that matter, disease.

### 6.4.3 Heritability and Inheritable

It is crucial to understand the term heritability and how it differs from the term inherited. Heritability is the degree to which differences between individuals are due to differences in genes. Heritability is expressed as the proportion of the variation among individuals with regard to a certain trait that is attributable to genetic rather than environmental variation (Falconer 1981). For example, differences between individual humans in height has a heritability index as high as 0.9 in some human populations (Bodmer 1976). This means that about 90% of the difference in height between individuals is due to genetic differences, and about 10% to differences in environment (e.g., nutrition, disease). However, this does not mean than any given individual's height is 90% genetic. The height of any individual is the result of an inseparable interaction of genes and environmental factors (Alcock 2001).

The difference between heritability and inheritable is crucial because a trait can be inherited regardless of its heritability. Highly heritable traits may be inherited; for example, a tall parent can have a tall offspring. However, a highly heritable trait may not be inherited.

The offspring of a tall parent may be short because of the environment in which the offspring develops (e.g., an environment lacking nutrition or in which disease is present). On the other hand, inheritance often occurs in the absence of heritability. Although two hands are normally inherited from one's parents, the number of hands one has is not a heritable trait – that is, there is essentially no genetic variance underlying hand number. In times past, hand number in humans was under strong selection, and that greatly reduced variation affecting the development of this trait.

### 6.4.4 Cultural

Although culture often is asserted to involve mental states, and sometimes to involve only mental states, culture becomes important in scientific studies when certain kinds of behavior or their consequences are observed. Most social scientists refer to culture when describing socially learned behavior (Flinn 1997), but what may appear to be essential to culture is not just that it is learned and shared – social– but that it is acquired from another individual and potentially transmittable to a third (Palmer 1997; Thornhill 2000; Coe 2003). To say that culture is socially learned behavior means only that the developmental causes of the behavior include, not that they are limited to, learning experiences involving other human beings. Speaking a language, for example, is clearly a cultural behavior, because the environmental influences leading to its occurrence include social learning. But the presence of another person is far from sufficient. Speaking a language only occurs when certain necessary genes have interacted with numerous environmental factors in addition to other people speaking the language.

If cultural is a term used to refer to socially learned and transmitted behaviors, then a culture would include a set of people who share a vast amount of socially learned behaviors. That is, the members of a culture share more socially learned behaviors with other members of that culture than they do with members of other cultures. If two cultures are geographically isolated, the cultural behaviors tend to be fairly distinct. However, in most cases, human cultures have been in contact and cultural behaviors are exchanged. Today it is probably more often likely to be true that the boundaries between cultures are at least somewhat arbitrary because of cultural behaviors being shared between what are designated as distinct cultures. In these situations "there will always be more than one way to cut out cultures from the fuzzy-edged clusters of habits that we observe" (Bruman 2006: 61). The term sub-culture is often used because within a culture some sets of individuals share more cultural behaviors with each other than they do with other individuals in their culture.

### 6.4.5 Traditional

Traditional refers to only those behaviors that are socially learned from a parent or other ancestor. Thus traditional behavior is a subset of cultural behavior and occurs when genes interact with many environmental factors, including a parent or other ancestor engaged in the behavior. Speaking a language, for example, is always cultural, but it may or may

not be traditional. An individual may have learned French at school, not from parents or grandparents. Speaking a language learned from someone other than an ancestor is not traditional; learning a language from an ancestor is traditional. Until recently, not only language, but nearly all other cultural behaviors were traditional.

Until recently, most shared socially learned behaviors, which were seen as characteristic of and as distinguishing a particular culture, were a consequence of social learning from common ancestors. The shared socially-learned behaviors defining a culture were traditional behaviors, and the members of the culture tended to be co-descendants, all of whom inherited culture from a common ancestor through grandparents and parents. This is why Kroeber, writing in 1923, observed that: "cultures are … inclined to be persistent …Even in times of the most radical change and innovation there are probably several times as many items of culture being transmitted from the past as there are being newly devised" (1923: 256–257). While this continues to be the case in many parts of the world in what we have come to call ethnic groups, the shared socially learned behaviors in other areas, such as in much of the US, are primarily due to social learning from people other than ancestors. In such areas, the shared socially learned behaviors defining a culture are often not traditional.

## 6.5 The Interactive View of Development and Health

The view that race correlates with health assumes that genes alone produce aspects of a phenotype that is correlated in some way with health. Race may be useful in studying health issues occurring in environments in which the health issues are highly heritable as well as inheritable, but only to the extent that the racial categories used correlate with the particular genetic differences related to the health issue. The fact that such correlation is often absent, because most genetic variation is found within a given racial category instead of between racial categories, weakens the relation between race and health. This approach is further weakened by the fact that genes alone do not create aspects of a phenotype that affect health. Even if certain genes are found to correlate with a racial category and the racial category correlates with a health issue, the health issue may not be causally related to those genes. The health issue may actually be related to some aspect of the environment (e.g., diet or exposure to pollutants) that also correlates with the racial category in that particular setting. These factors severely weaken the use of race as a variable in health research.

The typical view of culture is also limited by a focus only on the necessity of interaction with other people; the other factors, including genes, tend to be ignored. These other factors necessary for the development of cultural behaviors might be related to health conditions. Ignoring these other variables necessary to the development of cultural behaviors limits our ability to explain highly heritable health conditions. For example, without an understanding of the heritability of a health condition, merely detecting a correlation between a certain cultural practice and a health issue would not indicate whether or not the health issue was likely to be inherited. This is because the inheritance of the health condition by offspring might depend on inheriting both certain genetic factors and certain

environmental factors (e.g. diet) from the parents. To provide a clear example, a study conducted in Brazil of Leber's hereditary optic neuropathy (LHON) began by identifying four index cases from a remote area. Molecular analysis of blood showed that they were LHON, homoplasmic 11778, J-haplogroup. As these four individuals had an extensive family living in that rural area, 273 of the 295 family members were investigated (Sadun et al. 2002). The team conducted epidemiological interviews attempting to identify possible environmental risk factors and conducted comprehensive neuro-ophthalmological examinations, psychophysical tests, Humphrey visual field studies, fundus photography, and blood testing for both mitochondrial genetic analysis and nuclear gene linkage analysis. They found that the individuals were all descendants of an immigrant from Verona, Italy and that subsequent generations of his descendants demonstrated penetrance rates of 71, 60, 34, 15, and 9%. Age at onset ranged from 10 to 64 years of age. Current visual acuities varied from light perception to 20/400. The team was left unable to answer the question of why only some of the genetically affected individuals manifested the disease (Sadun et al. 2002). The obvious answer is that the expression of a disease is a consequence not only of the genes but of complex environmental factors.

Ethnicity is better able to explain health issues than are either race or culture because it incorporates both genetic and environmental (including cultural) factors. This is because ethnicity is defined by descent from common ancestors and involves the inheritance of both genes and culture. This is also why attempts to equate ethnicity with either genetic race or environmental culture have failed.

"(T)he notion that ethnicity has something to do with kinship or 'blood' is not new. Indeed descent seems to be, implicitly and very often explicitly, the essential element of the definition of those groups of 'significant others' that go under a wide variety of labels: tribe, band, horde, deme, ethnic group, race, nation, and nationality" (Van den Berghe 1981).

Ethnicity, when defined by descent, incorporates both genetic and cultural factors, including those factors related to health. The key to such an understanding is an appreciation of the importance of cultural traditions and an understanding of how they are inherited. Inheritance occurs "when and only when both genetic and environmental influences are repeated between generations" (Thornhill 2000). Traditions are a form of culture that is transmitted vertically, across generations from ancestors to descendants, parent to child. Traditions make up a significant amount of human behavior and distinguish the sets of co-descendants referred to as ethnic groups. Ethnic groups are distinguished by both genetic and environmental factors because traditions are inherited from one's ancestors when both the necessary genes and environmental factors are present.

In contrast to a race, an ethnic group is a set of co-descendants that cannot be identified solely by non-cultural phenotypes. Ethnicity is a category of co-descendants often identifiable only through particular traditional cultural traits. Typically these include language, clothing, tattooing, hair styles, dance, art, and other traits. In contrast to a race, an ethnic group is a set of people perceived to be co-descendants of a recent common ancestor (i.e., members share a more recent common ancestor with other members of the ethnic group than with nonmembers). An "ethnic group" is defined (at least implicitly) as sharing cultural traditions due to a common ancestry, and distinct from other categories of people on the basis of this asserted ancestry. An ethnic

group is perceived to be identifiable through traditional cultural markers, although their perceived common ancestry would imply that they have also inherited genes from their common ancestor.

The realization that ethnicity is a combination of genetic and cultural factors does not simplify the concept of ethnicity. Although ethnicity incorporates both genetic and cultural factors, it does not perfectly correlate with either. Descent from a common ancestor predicts that members of an ethnic group may be more likely to have a certain gene, but a correlation between genes and ethnicity is far from perfect. Each child inherits only 50% of our genes. While traditional behaviors identify one's ethnicity, not all members of an ethnic group will share all traditional behaviors. The amount of correlation between ethnic groups and both genes and traditions is also likely to vary from one ethnic group to another.

In the twenty-first century, we are less likely to find individuals who share genes due to common ancestry and who have maintained ancestral traditions. However, in the past, traditions remained unchanged for centuries or even millennia (Coe 2003). Although all forms of culture imply social interaction, traditions imply enduring social interactions between individuals sharing ancestry. The transmission of a tradition, such as learning how to make pottery, can require decades of social interaction during which strong social ties were formed and the history of the people was learned (Coe 2003). It is for this reason that anthropologists have seen traditions as embedded in social support (Corin 1995), as identifiers of group membership, and as very difficult to change without damaging important social ties (Coe 2003). Ethnicity is often communicated by using such things as distinctive cuisine and body decoration. Many ethnic groups have had explicit rules specifying that clan or tribal "brothers" or "sisters" were to be treated to some degree as if they were real brothers and sisters. One function of ethnic costumes and outfits seems to have been to identify, continuously and unambiguously, cooperative units – the ethnic groups. For much of human history, it would have been important to identify one's affiliation with certain others and dangerous to point out one's distinctiveness, or lack of membership. Farley recognized this separation when he wrote, "we are all ethnics; we represent some groupings of people who are or have been separate or different from other groupings of people" (Farley 1988: 2). Others have emphasized the importance of the we-they component of ethnicity when describing the "…unity that characterizes all ethnic groups… Despite differences, there is an overarching sense of 'we' (and of 'they') that emerges when collective fates and interests are at stake and when the larger group confronts outsiders" (Nagel 1996).

The failure to understand the vertical nature of culture led many sociologists to see culture as a product of horizontal social conformity acquired through proximity and readily subject to change. For this reason, sociologists predicted, especially in the decades after World War II, the demise of ethnicity. They felt that the horizontal social conformity they thought essential to ethnic identification would break down in industrialized, urban societies such as the United States (Park 1950; Wilson 1967; Bonacich 1980; Keefe et al. 1987). It was believed that ethnic groups would eventually disappear because technology is associated with urbanism that is, in turn, associated with mobility and the loss of community. Individualism (refusing to conform to others around you) and growing alienation (implied by this lack of conformity) would thus result in the loss of ethnicity.

While many of the traditional behaviors associated with ethnic groups have disappeared, it is important to recognize that identification with ethnic groups has not.

A number of studies conducted in the second half of the twentieth century have shown maintenance of or increase in ethnic identification despite a historical loss of traditions and weakening of mechanisms that protected ethnic boundaries (e.g., rules of endogamy; loss of ancestral language) (Scupin 2003). For example, American individuals of Irish descent continue to identify with their ancestry despite the fact the family may have been in America for generations and the descendants have never traveled to Ireland. Similarly, many descendants of Newfoundlanders who have migrated to Alberta continue to emphasize their Newfoundland heritage despite having never been to Newfoundland. While many traditional behaviors that once distinguished ethnic groups have disappeared, the ethnic wars now occurring around the world demonstrate that ethnicity, including the strong passions associated with in-group membership and the antagonism directed against outsiders, remains (Coe 2003; Scupin 2003).

Ethnicity is further complicated by the fact that there are nearly an infinite number of common ancestors that could be used to delineate an ethnic group. A focus on more distant common ancestors identifies a larger ethnic group because it implies a larger set of co-descendants, while a focus on a more recent common ancestor identifies a smaller ethnic group (Palmer 1997). All mammals, for example, share a common ancestor, although that ancestor is quite distant. All humans share a closer common ancestor with each other than with other mammals. A Hopi shares a more recent common ancestor with other Hopi than with members of other Pueblo tribes. However, a Hopi shares a more recent common ancestor with members of other Pueblo tribes than with members of Athabascan tribes, such as the Navajo and Apache. A Hopi may share a more recent common ancestor with other American Indians, including the Athabascans, than with those of European descent (Dillehay 2001). Humans routinely expand or contract their ethnic category by focusing on more distant or nearer common ancestors in different situations. For example, a person may be a Lakota in one situation and a Native American in another.

The use of cultural behaviors and their products to identify ethnicity also complicates the concept of ethnicity. Individuals can manipulate their ethnicity by manipulating their cultural behaviors. Sociologists have noted that the ethnicity claimed by an individual could change depending on the social situation (Nagata 1974; Cohen 1978; Okamura 1981; Nagel 1996). Individuals, particularly when they have mixed ancestry as is the case among most Americans, can identify with different ethnic groups at different times, but there are limitations. As van den Berghe points out, “the fiction of kinship, even in modern industrial societies, has to be sufficiently credible for ethnic solidarity to be effective. One cannot create an instant [ethnic group] by creating a myth. The myth has to be rooted in historical reality to be accepted. Ethnicity can be *manipulated* but not *manufactured*” (Van den Berghe 1981). The tie of ethnicity to at least a plausible approximation of ancestry is what makes ethnicity a more powerful concept than culture.

The importance of the interactive view of development when applied to health is illustrated by Alcock, who writes:

> “… every visible attribute of every organism is the product of a marvelously complex and all-pervasive interaction between genes and environment. The evidence for the interactive theory of development is overwhelming, but a nice illustration of the point comes from work showing that persons with different genes can develop similar traits given the appropriate

> environments. A famous example of this sort comes from studies of a human gene we will label PAH.… [I]ndividuals with certain alleles of the PAH gene make forms of phenylalanine hydroxylase that may fail to do their job properly. Persons carrying these variant genes are generally unable to convert phenylalanine to tyrosine and therefore phenylalanine typically builds up in their cells. The extra phenylalanine [results] in the formation of considerable amounts of… [phenylpyruvic acid], which happens to be developmentally damaging in large quantities… [with] the sad result [being]… a child who suffers from severe mental retardation" (Alcock 2001).

Because this form of mental retardation is influenced by a particular gene, individuals unaware of the interactive view of development tend to only focus on this particular factor, calling it a genetic disease that is innate in those who have it. This view is inaccurate because it ignores the other factors involved in the development of the mental retardation.

Because of our recently acquired understanding of the interactive view of development,

> "today any newborn testing positive for phenylketonuria is immediately placed on a highly restrictive diet very low in phenylalanine. This intervention does not change the genes of the babies, but it does change the chemical environment of the brain cells, and thereby helps prevent the buildup of phenylalanine and its devastating by-product, phenylpyruvic acid. As a result, brain cell development usually proceeds more or less normally, as does intellectual development. Thus, having certain alleles of the PAH gene does not condemn one to be mentally retarded. The disease is not genetically determined…" (Alcock 2001).

## 6.6 Identifying Ethnicity Using Proxy Measures

Another advantage to a clear understanding of ethnicity is that it reveals limitations to some of the various ways that are currently used to identify a person's ethnicity. Based on the assumption that culture is acquired horizontally, general geographic origin (e.g., Cuban American, Asian American) was assumed to be a good proxy measure for ethnicity (Ortiz and Arce 1984; Kato 1996). We often continue to see geography associated with ethnicity and race. Patrinos wrote in a recent edition of *Nature Genetics*: "With very rare exceptions, all of us in the US are immigrants. We bring with us a subset of genes from our homelands, and for many Americans, often first-generation but more commonly second-generation, the plural noun 'homelands' is appropriate" (Patrinos 2004). However, a general geographic place, such as a country, often contains numerous ethnic groups with distinct traditions. For example, the category of Native American contains hundreds of distinct tribes. Limiting geographic origin to a smaller area is helpful, although it does not solve the problem in the case of multiple ethnic groups who inhabit a geographic area.

Other proxy measures for ethnicity are language and surname, but these are also problematic. In the 1930s, the US Census Bureau began to look at Hispanics, persons of Spanish/Hispanic origin, as a separate group (Miranda 1997). An early policy in the southwestern states was to group together individuals with Spanish surnames. This practice was abandoned when a study conducted by the US Census Bureau indicated that about one third of those who claimed Spanish descent did not have Spanish surnames, and around a third of those with Spanish

surnames did not claim Spanish descent (United States. Bureau of the Census. 1973). While in the US individuals frequently inherit the last name of their fathers, those individuals may be likely to identify with maternal traditions and ethnic identity. Some surnames are shared with other ethnic groups, such as the Spanish surname Miranda, which is shared by Italians, Portuguese, Filipinos, and Brazilians (Miranda 1997). Language as a proxy measure for ethnicity would lead us to omit individuals who do not speak the language of their ethnic group. For example, a Hispanic individual may or may not speak Spanish. Many entire ethnic groups in the US have lost their traditional language, but continue to identify with the ethnic group.

Failure to define the term ethnicity explicitly and empirically has led to an inability to identify why and how ethnicity might be related to differential health outcomes, and even to the argument that ethnicity may not be an important variable in health research. It is crucial to remember that ethnicity is based on ancestry, and that any number of traditional cultural behaviors may be used to identify a person with that ancestry. This approach facilitates the identification of correlations between ethnicity and health as it focuses attention on any number of possible traditions that might influence health.

## 6.7 Ethnicity and Health

The realization that ethnicity is a combination of genetic and cultural factors does not simplify the relationship between ethnicity and health. It does, however, direct research toward better ways to deal with the complexities of this relationship due to the focus on the inheritance of both genes and traditions that occur in the development of offspring. This makes the concept of ethnicity extremely powerful in sorting out the various genetic and environmental factors that influence individuals and their health. Consider a population where there is a correlation between a health issue and ethnicity. An understanding of what ethnicity is, and of the interactive theory of human development, allows an efficient approach to identifying the genetic and environmental factors involved in the health condition.

If there is a correlation between ethnicity and the health condition, it could mean that the condition is more common within the ethnic group for a number of reasons. The correlation could be due to genes inherited by the recent common ancestry of the ethnic group, the cultural traditions inherited by the recent common ancestry of the ethnic group, or due to some other variable that just happens to correlate with the ethnic group (e.g., residence near a toxic waste dump, socioeconomic class). Differences in health outcomes and in the behaviors that influence those outcomes may be artifacts of the tendency for certain ethnic groups to share a common socioeconomic status (Landry 1987). This realization directs investigators towards the tests needed to determine exactly which factors are related. In addition to genetic testing, variation in traditions within the ethnic group could be examined to determine which factors may be causal.

A number of anthropologists have identified traditions that act like “meta-traditions” (Todd VanPool, personal communication, June 20, 2007) because they function to preserve other traditions necessary for the persistence and well-being of groups, and hence, likely

to be related to some aspect of health. Meta-traditions, such as religion, often affect health in numerous ways.

"Cultural influences on health and medical care involve such basic aspects of human behavior and belief systems as religious practices, language, folk medicine, diet, dress, norms and values, and help seeking behavior. These cultural practices in turn have an impact on perceptions of symptoms, definitions of illness, delivery of health services, disease prevention, health promotion, medical practice and patient adherence" (King and Williams 1995).

Health outcomes are not only influenced by culture, but also by discrimination by the dominant culture, self-imposed isolation, physical environment, resources available, socioeconomic and political factors, limitations of health care (including provider ignorance of the sociocultural determinants of health and disease), or genetic factors (Farley 1988).

Some ethnic groups around the world have maintained a unique group identity despite intense contact with other cultures. For example, the Amish of North America put so much emphasis on maintaining their traditions that they are known as the "People of Preservation" (Ruth 1991). Anthropologists have argued that the cultural features crucial to persistence include common "language, style of dress and adornment, religion, patterns of social interaction, and food habits" (Crews and Bindon 1991), as well as the close kinship ties that are necessary for and resulting from the transmission of traditions (Coe 2003). Ethnic clothing often functions to continuously and unambiguously identify ethnic or ancestral groups. We know that a member of the Tsachilai tribe is a Tsachila by the manner of their dress, body paint, and even their hair arrangement (Coe 2003). This body decoration is inherited from one's ancestors, identifies one as a descendant of that ancestor, and identifies others who share that ancestry and with whom each must cooperate. Even today in the US, individuals who cooperate (e.g., sorority sisters, gang members) tend to dress similarly and often claim metaphorical ancestry. Dietary traditions have been promoted as an important tradition that persists long after other aspects of culture are no longer evident (Van den Berghe 1981). Food-sharing rituals persist because they involve family and reinforce the important kinship ties that form the basis of social support (Van den Berghe 1981).

The importance of tradition is evident in the American Indian populations of the United States. American Indian tribes, as members of unique and diverse ethnic groups, tend to each have rules encouraging respect for elders (e.g., honor your elders who are the bearers of traditions) and ancestors, who are the creators of tradition. Such respect may be a meta-tradition, necessary for the successful transmission of tradition, including those related to health, and for building strong social relationships. The giving of respect to elders is also paramount among the Amish: "Wisdom accumulates with age, and with age comes respect. Old people retain the respect of children and grandchildren. Obedience to parents is one of the most common themes in Amish preaching… Since the wisdom of the aged carries more weight than the advice of younger men, the conservation of the entire community is assured and the religious ideals are protected from too much change" (Hostetler 1993, 14–15). Although male elders have at least more formal power among the Amish than do female elders, mothers are particularly important in Native American societies, as families and extended families revolve around them and their children (Coe 2003). "So important are mothers that even very ferocious people…would never hurt a mother because the mother is considered the fountain of kinship" (Briffault 1931).

Religion, which in the past was often inherited from one's ancestors through one's grandparents and parents (Steadman and Palmer 1995), is also often associated with rules specifying appropriate kinship behavior. Of importance to the field of cancer prevention, religion and beliefs "are at the core of preventive and curative health practices" (Airhihenbuwa 1995). Healthful behaviors are learned from ancestors via close kin and result in an obligation owed to one's ancestors. Religions around the world promote kinship behaviors that make the transmission of traditions possible. Christians, as one example, encourage individuals to honor their parents and care for their children. This rule, however, is not unique to Christianity. Kinship is the informal hierarchy used for teaching the young and reminding adults of culturally appropriate behavior. Storytelling, along with modeling and guided learning, is the method most often used to teach traditions. Humans respond to stories and they influence behavior (Coe 2003; Coe et al. 2005; Coe et al. 2006).

Some of the evidence of the relationship between traditions and health are the consequences of losing these traditions. Rapid loss of cultural traditions, whatever the cause, can leave populations vulnerable to certain health problems (Swedlund and Armelagos 1990; Corin 1995; Coe 2003). A loss of traditions, particularly those encouraging kinship behavior, may be associated with an increase in high-risk behaviors, including substance abuse and failure of a pregnant woman to protect her health and the fetus (Coonrod et al. 2004).

## 6.8 Applications to Disparity Research

Cancer health disparities in ethnic minorities are a serious problem in the United States. If we recognize that ethnicity is inherited and refers to categories of individuals who are co-descendants of a common ancestor, we may begin to understand its importance and the role it may play in both health and research. Ancestors are a key to understanding ethnicity, yet it is important to determine the breadth of how ancestry is defined. The guidelines of the 1990 census recognize the importance of ancestry and inheritance of ethnicity. "A person is of Spanish/Hispanic origin if the person's origin (ancestry) is Mexican, Mexican-American, Chicano, Puerto Rican, Cuban, Argentinean, Colombian, Costa Rican, Dominican, Ecuadorian, Guatemalan, Honduran, Nicaraguan, Peruvian, Salvadorian, from other Spanish-speaking countries of the Caribbean or Central or South America, or from Spain" (United States. Bureau of the Census. 1990).

Although ancestry is included in this definition, the term Hispanic refers to a very heterogeneous population. Hispanics share only a very distant ancestors, perhaps one that lived tens of thousand of years ago, because the ancestors of Hispanics came from indigenous populations spread across the Americas, as well as from populations living in Europe, Asia, and Africa. Some Hispanic ancestors, like those of American Indians, came hundreds or even thousands of years ago from different and diverse ecological habitats. These ancestors brought distinct cultural behaviors that underwent unique adaptations in response to the environment encountered in the New World. These practices were blended with older ones to produce a rich and highly diverse cultural fabric. Today, through the

process of acculturation to a more western lifestyle, many of these traditions are rapidly disappearing. Hispanic is an extremely complex term that has historical, social, cultural, legal and political connotations and ramifications.

It is not clear how much of the variance in health outcomes can be explained by unique genetics and cultural practices attributed to such a diverse ethnic category. There are regional patterns and socioeconomic patterns to disease incidence that may or may not be associated with ethnicity. Not unrelated is the fact that in general, ethnic minorities face greater barriers obtaining access to medical care (De la Cancela 1992; Hale 1992). Lack of access to health care providers means not only that diagnoses are underreported, but also that diseases are diagnosed at later and more serious stages. Nevertheless, by recognizing that ethnicity is related to descent, we gain a great deal of understanding. The proper focus on individuals sharing common descent has a genetic component (genes are inherited from ancestors) as well as cultural components (traditions are inherited from ancestors).

Many of the problems currently faced in health research disparities are related to our current inability to understand ethnicity and its importance. Researchers have often used the wrong frame of reference. In so doing, researchers have placed themselves in the position of trying to understand ethnicity from the standpoint of a period of time in which intermarriage, migration, and westernization have led to ethnic groups in which the members have few, if any, cultural traits in common.

For this reason that Fullilove criticized health research for focusing on "small periods of time…[e]ven longitudinal studies are limited to 40 or 50 years. The evolution of human behavior and ecosystems within which it is located must be understood from the perspective of much longer time frames" (Fullilove 1998). Ethnicity, as van den Berge explains, has to be validated by several generations of common historical experience (Van den Berghe 1981, p. 16).

By focusing on ethnicity as a descent category that develops over time we can turn this complex concept into a powerful means of identifying both the genetic and environmental (including cultural) factors influencing health. However, this will only be possible if political agendas are kept separate from the scientific study of ethnicity and health. This represents a considerable challenge because as ethnicity is associated with in-group and out-group identity, it has always been political, associated with judgments of good and bad, appropriate and inappropriate. In 1923, Kroeber cautioned anthropologists, warning them that discussions of race were very likely to be guided by "feeling, usually of considerable strength, which tends to vitiate objective approach" (1923: 205). For some researchers and many lay people, ethnicity is pejorative, a name given to minority populations by a "dominant" society. For these individuals, the established race and ethnic classifications or taxonomies in American societies evolved from systems of stratification, power, and ideology (Amick 1995). In this setting, certain populations are seen as marginal, dismissed as "ethnics" because they lack political power and have a low social status (Amick 1995). For others, ethnicity is a positive, "Diversity and ethnicity are basic to our species. Many of us want to be different in belief, looks and actions from others…" (Farley 1988: 1). The positive nature of ethnicity may be implied in the way people continue to identify with ethnic groups, long after identifying features have been lost and boundaries erased and even when faced with prejudice for such an identification.

Both of these positions, however, may be guilty of the naturalistic fallacy. From a scientific point of view, ethnicity is neither good nor bad, it just is. Cross-cultural judgments

that conclude that some ethnic groups are somehow less or better than others, are simply judgments. To the extent that such judgments affect health and access to health services they are examples of racism or ethnocentrism. Clearly, while this political aspect of ethnicity should be the focus of study, we should recognize that if we are to understand the fundamental meaning of ethnicity, we have to appreciate that there are, inherently, no differential values related to ethnic group membership. All of our ancestors, evolutionarily speaking, were equally successful. They all left behind them a line of descendants that began with the origins of life on earth and continues until today. Ethnicity and race as a political category have unfortunately led to claims that current racial and ethnic designations have little relevance to science and are essentially pragmatic or politically expedient categories (Weissman 1990; Hahn 1992; Hahn et al. 1992). Ethnicity, however, is much more.

## 6.9 Conclusion

Ethnicity cannot be understood as a term useful in scientific study until its necessary elements (genes, environment, heritability, inheritability, culture and traditions) are understood. Once we understand that all these elements play a role in the disease process, we can begin to move forward in the study of the relationship between ethnicity and disease. Both the genetic and environmental backgrounds are important. The field of genomics now makes it possible to study the underlying mechanisms of human health in relation to diet and other environmental factors (Desiere 2004). However, the environment is more than drugs and toxic pollutants; it is complex and involves social relationships and traditional behaviors.

Traditions do not only serve as in-group and out-group identification. The process of transmitting a tradition from one generation to the next builds the social relationships that are crucial to health. Health behaviors, today as in the past, are by and large taught in families. Family members are present to reinforce these behaviors. Kinship is centered on a maternal-child relationship that is characterized by the responsibilities the one at the top of the hierarchy, the mother, has to those beneath. Lay health worker programs, which appear to be effective in promoting health, are built on this basic maternal-child, duty-responsiveness model (Staten et al. 2004). However, more than a benevolent hierarchy is implicated in promoting health behaviors. We ignore families in our health promotion efforts at our peril because families reinforce behaviors in a myriad of ways.

Finally, programs that interest ethnic minorities and non-minorities should be developed based on the methods used in traditional societies to educate the young and to reinforce beliefs among adults. Cultures are holistic; education takes place during all activities of daily living. Learning incorporates the humanities, the spiritual and the pragmatics of subsistence strategies for procuring food. Those from whom we learn are those we trust. Learning traditionally has not been done in a classroom setting but involves modeling and guided practice. A lecture approach to health will never be attractive to most people who have been raised in a rich cultural environment.

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# Complementary and Alternative Approaches to Cancer Prevention

# 7

**L.K. Larkey, H. Greenlee, L.E. Mehl-Madrona**

Complementary and alternative medicine (CAM) includes a variety of therapeutic approaches not typically taught in conventional medical schools or used by the majority of conventionally trained physicians (Gordon and Curtin 2000). Complementary refers to modalities used to complement, that is, used in addition to conventional medicine, while alternative is usually used to describe treatments intended to replace conventional treatment (Murphy et al. 1997). Some such practices enjoy a history of research to support claims of efficacy but have not gained popularity among the majority of medical practitioners (e.g., use of acupuncture to reduce chemotherapy-induced nausea) (Dibble et al. 2000; Mayer 2000; Shen et al. 2000); others either lack or have unsupportive research to back claims of safety or efficacy. Even so, the determination of which modalities are complementary or alternative may shift over time as research and practice move some into conventional use and disprove others. Some practices less amenable to our current research epistemology may never move out of CAM nomenclature or perceptions (such as multi-modality system approaches, energy medicine, or spiritually-based practices).

Currently, CAM broadly encompasses a range of substances, practices, practitioners, and belief systems that fall outside of the conventional medical model. CAM substances include botanicals (also known as herbs), vitamins, minerals, specific nutrients, enzymes, foods, and homeopathy. CAM practices include yoga, Qigong, meditation, dietary modifications such as macrobiotic or raw food, and numerous culturally-based interventions focusing on various combinations of body, mind or spiritual interventions. CAM practitioners use therapeutics with varying levels of training, with great variation in licensing laws and insurance reimbursement depending on state (United States), province (Canada) or country (Cherkin et al. 2002; Eisenberg et al. 2002). These practitioners include, but are not limited to, naturopathic physicians, chiropractors, acupuncturists, herbalists (both Western and Chinese), massage therapists, Ayurvedic practitioners, Native American healers, Tibetan physicians, Reiki practitioners, Healing Touch practitioners, and spiritual healers. Although conventional in many cultures, belief systems or perceptions of health, healing and the body that are considered to be CAM by conventional medical practitioners in the United States (US)

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include Traditional Chinese Medicine (TCM), Ayurvedic medicine, Tibetan medicine, and Native American medicine. CAM substances, practitioners, practices, and belief systems have undergone varying levels of scientific scrutiny, with the current emphasis in US research focusing on elucidating their biological mechanisms of action.

The US National Center for Complementary and Alternative Medicine (NCCAM, 2007) at the National Institutes of Health (NIH) categorizes CAM into four domains: mind–body medicine, such as meditation, prayer, and music therapy; biologically-based practices, such as dietary supplements, herbal products, and functional foods; manipulative and body-based practices, such as chiropractic manipulation and massage; and energy medicine, which includes biofield therapies such as Qigong, Reiki, and Therapeutic Touch, and bioelectromagnetic-based therapies, such as pulsed fields and magnetic fields. In addition, NCCAM studies whole medical systems, which cut across all domains, including naturopathic medicine, TCM, and Ayurveda.

This chapter begins by discussing how CAM therapies currently fit into the field of cancer prevention research and then discusses some of the more promising CAM biological agents being studied for their cancer prevention properties, including foods, spices and botanicals. The research on these biological modalities most resembles conventional research on mechanisms of action, such as chemoprevention or dietary interventions that effect biochemical changes. Next, this chapter discusses some of the CAM approaches that are less well matched to the medical model of biochemical responses, including healing approaches that utilize the mind–body relationship; traditional systems of healing founded in ancient wisdom and practice; and the more esoteric areas of spirituality and energy medicine.

## 7.1 CAM and Cancer Prevention Research

Although there are no proven methods to definitively prevent cancer in either conventional medicine or CAM, many cancer prevention approaches currently under investigation focus on dietary changes, nutritional supplements, lifestyle modifications (e.g., increasing exercise, decreasing sun exposure), and decreasing environmental exposures, many of which fall into the field of CAM research.

Although the field of research on CAM in treatment of diseases, including cancer, has exploded in the past few years, there is a notable lag in the development of research protocols in the area of cancer prevention. Research on treating disease generally progresses through systematic phases of theory development, lab and animal testing, then small pilot trials to large population studies (see Chap. 1). However, prevention research may follow a less convenient trajectory. Theories of cancer prevention often originate from correlational, longitudinal databases or observations of populations, national or cultural patterns of diet or lifestyle that might impact cancer rates (Krishnaswamy 1996). Following epidemiologic investigation, potential mechanisms for affecting the precursors or biomarkers of cancer development (and those associated with prevention of recurrence when applicable) are studied. Trials to investigate the effects of manipulating such variables and eventually measuring cancer outcomes in CAM have yet to take a prominent role in the US. We have just begun

to see the results of large-scale national trials in conventional chemoprevention (e.g., BCPT, The Breast Cancer Prevention Trial; STAR, the Study of Tamoxifen and Raloxifene) (Smith 2003; NCI 2006; Fisher et al. 1998) and in nutritional interventions such as the Carotene and Retinol Efficacy Trial (CARET) (Omenn et al. 1996), WINS (the Women's Intervention Nutrition Study) (Chlebowski et al. 2006) and WHEL study (Women's Healthy Eating and Living study) (Thomson et al. 2007). It will require funding opportunities for preliminary research to investigate CAM to demonstrate its effects on biomarkers before such large investments will be made for research in CAM in cancer prevention in the US.

Cancer patients and survivors have increasingly turned to CAM approaches for treatment and alleviating the side effects of treatment. Even a decade ago, in a systematic review, Ernst and Cassileth (1998) estimated that one-third of all cancer patients in North America and the United Kingdom (U.K.) used CAM. The increasing and continued move toward CAM among cancer patients makes it even more important for researchers to test safety and effectiveness of these alternatives (Bernstein and Grasso 1983; Cassileth and Deng 2004). Over the past few years, the term "integrative oncology" has been increasingly used to describe clinical settings or research approaches that combine the best of conventional cancer care with evidence-based CAM practices. This approach is largely based on the biomedical model, which allows some aspects of CAM to be more easily integrated into conventional care. Many of the modalities used focus on symptom relief during cancer care. However, some of them also focus on preventing cancer recurrence by strengthening the immune system or interventions having direct apoptotic effects (Gordon and Curtin 2000).

The use of CAM in cancer prevention has not been thoroughly evaluated despite growing evidence that the public is utilizing a wide range of practices and products with that intent. In 1990, it was estimated that 425 million visits to CAM providers were made in the US (as compared to 388 million visits to conventional caregivers), and that CAM expenditures for that year in the US amounted to approximately $13.7 billion (Eisenberg et al. 1993). Estimates for out-of-pocket expenses rose to $27–34 billion by 1997 with a "staggering 629 million visits to alternative practitioners" in the US (Neal 2001). Specific to cancer prevention, a recent study showed that 55% of sampled men with a family member diagnosed with prostate cancer were using some form of CAM and 30% were using supplements specifically purported to be prostate-specific cancer prevention agents (Beebe-Dimmer et al. 2004).

## 7.2 Botanical Agents: Foods, Spices, and Herbs

CAM cancer prevention strategies that employ botanical agents are often directed towards specific physiological pathways. These agents may include foods, spices, botanicals, or specific nutrients. Possible chemopreventive effects of these agents may be due to their antioxidant, immune modulating, hormone modulating, anti-angiogenic, apoptotic, or anti-metastatic properties. Many of these properties have been described in detail elsewhere (Boik 2001; Park and Pezzuto 2002). Some of the most promising candidates for further exploration, according to the existing literature, are summarized here.

The definitive line between a food, a spice and an herb is easily blurred depending on how substances are prepared or formulated. For example, garlic may be considered a food, a flavoring or an extract. In general, a food is considered something that is eaten as a primary source of nutrition. Much of the food chemoprevention literature focuses on dietary approaches that encourage eating high amounts of fruits, vegetables and whole foods (i.e. foods that have not been commercially processed). Cruciferous vegetables, fiber and soy are currently under investigation for activity in cancer prevention. Other dietary research efforts in cancer prevention have focused on the benefits of specific diets, such as a macrobiotic diet (Kushi et al. 2001). Micronutrient and dietary interventions for cancer prevention are addressed in more detail in Chap. 3 of this book.

Spices are the aromatic parts of plants that are used as seasonings, rather than for nutrition. Some spices, as well as many foods, contain concentrated amounts of phytochemicals (naturally occurring biochemicals that give plants their color, flavor, smell, and texture) that may have preventive properties (Polk 1996; Nishino et al. 2000). Many of these properties have been demonstrated in vitro and in preclinical or animal studies, with only limited data in humans (Lampe 2003). Antioxidants, which help prevent free radical formation and damage, are found in cloves, cinnamon, oregano, pepper, ginger, garlic, curcumin, coriander, and cardamom (Wu et al. 2004). Biotransformation enzymes, which metabolize chemical carcinogens, are induced by coriander, curcumin, cinnamon, and *Wasabia japonica* (Japanese domestic horseradish). Garlic, cloves, cinnamon, chili, horseradish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, onion, and, celery have all demonstrated antibacterial activity. Ginger and curcumin have anti-inflammatory properties. Thus, many of these spices demonstrate active properties that are potentially cancer preventive.

Botanicals, more commonly known as herbs, may be fresh, dried, freeze-dried, juiced, or extracted. The whole or specific parts of the plant may be used, including the leaf, flower, stem or root. Some of the cancer-related research on botanicals to date focuses on the role of botanicals to treat cancer (e.g. mistletoe extract) (Zarkovic et al. 2001). However, an increasing amount of research is focusing on botanical agents for their chemopreventive effects.

The specific agents reviewed below provide examples of the various mechanisms being investigated in botanical chemoprevention research: curcumin for its antioxidant and anti-inflammatory properties; flaxseed for its hormone modulating effects; mushrooms for their immune modulating properties; and ginseng for its immune modulating and adaptogenic effects. It is important to note that these and other herbal agents have active substances that may interact with other herbs, supplements or medicines and may have unexpected side effects. Herbal products that modulate certain enzymes or proteins may have adverse interactions with anti-cancer medications (e.g. ginko, echinacea, ginseng, St. Johns wort, kava) (Sparreboom et al. 2004). All foods, spices and herbs used for medicinal purposes should only be taken under the care and supervision of a practitioner highly trained in the area of botanical medicine.

### 7.2.1 Curcumin

Curcumin, an extract of turmeric (*Curcuma longa*), has potent antioxidant and anti-inflammatory effects and has been studied as a chemopreventive agent for many cancer

tumor sites. Pre-clinical studies of curcumin have demonstrated its ability to inhibit carcinogenesis in breast, cervical, colon, gastric, hepatic, leukaemia, oral epithelial, ovarian, pancreatic, and prostate cancer models (Aggarwal et al. 2003; Aggarwal and Shishodia 2006). Multiple molecular targets have been identified, including, but not limited to NF-κB (nuclear factor-kappa B), AP-1, STAT3, Akt, Bcl-2, Bcl-$X_L$, caspases, PARP [Poly (ADP-ribose) polymerase], IKK (Ikappa B kinase), EGFR (epidermal growth factor receptor), HER2 (human epidermal growth factor receptor 2), JNK (Jun N-terminal Kinase), MAPK (mitogen-activated protein kinases), COX2 (cyclooxygenase 2), and 5-LOX (5-lipoxygenase). Curcumin has also been shown to inhibit phase I enzymes and induce phase II enzymes, such as glutathione-S-transferase (Sharma et al. 2005). To date, some of the most promising effects have been demonstrated in colon cancer models (Johnson and Mukhtar 2007). Five phase I trials have shown the safety and tolerability of curcumin in colorectal cancer patients using doses of up to eight grams per day. Phase II trials are currently examining curcumin's effect in primary and secondary colon cancer prevention (see NIH, National Institute of Health's website on cancer clinical trials).

### 7.2.2 Green Tea

The polyphenols contained in green tea (*Camellia sinensis*) are potent antioxidants. One of the most abundant polyphenolic compounds, (-)-epigallocatechin-3-gallate (EGCG) is receiving a great deal of research attention for its chemopreventive effects, including antioxidant, antiangiogenic, and apoptotic properties. To date, preclinical studies have identified specific effects of EGCG including regulating expression of VEGF (vascular endothelial growth factor), matrix metalloproteinases, uPA, IGF-1 (insulin like growth factor 1), EGFR, and cell cycle regulatory proteins (Shankar et al. 2007). In addition, EGCG inhibits NF-κB, PI3-K/Akt, Ras/Raf/MAPK and AP-1 signalling pathways. Animal and observational data suggest that green tea consumption may lower blood estrogen levels, thus lowering breast cancer risk, while limited data suggest that black tea may increase breast cancer risk (Wu and Yu 2006). Currently, phase I and phase II EGCG chemoprevention studies are investigating its effects on breast, skin, lung, prostate, cervical, and bladder cancers, as well as in chronic lymphocytic leukemia and in Barrett's esophagus.

### 7.2.3 Immune Modulating Mushrooms

Several mushrooms have been identified as having immune modulating effects (Pelley and Strickland 2000). Three mushrooms, *Coriolus versicolor*, *Ganoderma lucidum* (reishi or lingzhi), and *Grifola frondosa* (maitake) contain polysaccharides with particularly promising immune modulating effects that may be effective in primary and secondary cancer prevention. *Coriolus versicolor* had historically been used in Traditional Chinese Medicine (TCM) and is frequently used in Japanese oncology settings. *Coriolus versicolor* is usually used in extract form, either as protein-bound polysaccharide-K (PSK) or polysaccharide-P

(PSP). Recent research has shown immune-stimulatory actions of these polysaccharides on T lymphocytes, B lymphocytes, monocytes/macrophages, bone marrow cells, natural killer (NK) cells, and lymphocyte-activated killer cells as well as promoting the proliferation and/or production of antibodies and various cytokines such as interleukin (IL)-2 and IL-6, interferons, and TNF (tumor necrotic factor) (Chu et al. 2002; Fisher and Yang 2002). Preclinical studies of *Ganoderma lucidum* have shown inhibitory effects on NF-κB, AP-1, uPA and uPAR (Sliva 2003). Preliminary clinical trials have shown some beneficial effects on immune function in patients with colon cancer (Chen et al. 2006) and lung cancer (Gao et al. 2005). Maitake mushroom (*Grifola frondosa*) is an edible mushroom that is also under investigation for its anticancer properties. The most active ingredients in this mushroom appear to be polysaccharides 1,3 and 1,6 beta-gluca). These constituents are marketed in the form of proprietary D- or MD-fraction extracts. Maitake extracts have been shown to enhance bone marrow colony formation in vitro (Lin et al. 2004), induce apoptosis in prostate cancer cells (Fullerton et al. 2000), decrease the effective dose of mitomycin-C in tumor-bearing mice (Kodama et al. 2005), and activate NK cells in cancer patients (Kodama et al. 2003).

### 7.2.4 Ginseng

The concept of "adaptogen" was first proposed by Soviet scientists in the late 1950s, suggesting that an adaptogen is any substance that exerts effects on both sick and healthy individuals by 'correcting' any dysfunction(s) without producing unwanted side effects (Davydov and Krikorian 2000). Several botanicals are purported to have adaptogenic effects that provide resistance to stress and fatigue, with various ginsengs meeting these criteria. The chemistry of the secondary compounds and pharmacological effects of *Eleutherococcus* (Siberian ginseng) isolated thus far support the hypothesis that the reported beneficial effects of such adaptogens derive from their capacity to exert protective or inhibitory action against free radicals. Of the compounds isolated from Siberian ginseng, six compounds show various levels of activity as anti-oxidants, four show anti-cancer action, three show hypocholesterolemic activity, two show immunostimulatory effects, one has choleretic activity, one has the ability to decrease/moderate insulin levels, one has activity as a radioprotectant, one shows anti-inflammatory and anti-pyretic activities, and yet another has shown activity as an antibacterial agent (Davydov and Krikorian 2000). Some of the compounds show more than one pharmacological effect and some show similar effects although they belong to different chemical classes.

The most active components of another ginseng, Panax ginseng (also known as Asian or Korean ginseng), are ginsenoside saponins (Tyler 1993). There are approximately 30 different saponins that have been isolated from Panax ginseng; a number of these have been evaluated for their potential use in cancer prevention (e.g. ginsenoside $Rg_3$, $Rg_5$, $Rh_1$, and $Rh_2$) (Yun et al. 2001b). The quantity of ginsenoside in roots varies by type and age of the plant. Ginsenosides have been shown to stimulate the immune system and inhibit cancer cell proliferation (Block and Mead 2003; Shin et al. 2000; Xiaoguang et al. 1998). Preliminary in vitro and epidemiologic evidence show non-specific preventive

effects in multiple tumor sites (Shin et al. 2000; Yun 2001, 2003; Yun et al. 2001a,b; Chang et al. 2003). Ginseng has also demonstrated anti-inflammatory properties which target many of the important pathways in the inflammation-to-cancer sequence (Hofseth and Wargovich 2007). Early clinical trials have shown some benefit in improving subjective quality of life reports in cancer patients (Kim et al. 2006). An ongoing NIH study is investigating the effect of ginseng on cancer-related fatique, which may have an effect on longer-term cancer outcomes.

### 7.2.5 Flaxseed

Flaxseed is rich in lignans (a type of phytoestrogen) and fiber. Lignans may have chemoprotective properties in breast cancer due to their inhibition of estrogen production; they have been shown to inhibit growth of human mammary tumor cells (Hirano et al. 1990), reduce mammary tumor initiation (Thompson et al. 1996), stimulate sex hormone binding globulin, which binds estrogens that increase cancer risk (Adlercreutz et al. 1992), and to inhibit aromatase activity, which then decreases endogenous estrogen level (Adlercreutz et al. 1992, 1994). More recent studies have focused on how flaxseed influences endogenous hormone concentration and urinary estrogen metabolites associated with increased cancer risk. Flaxseed in the diet has been shown to significantly reduce serum concentration of 17-beta-estradiol and estrone sulfate (Hutchins et al. 2001). Flaxseed supplementation significantly increased urinary 2-hydroxyestrone excretion and increased the 2:16 alpha-hydroxyestrone ratio in studies of pre-menopausal (Haggans et al. 2000) and post-menopausal women (Haggans et al. 1999). Flaxseed's influence on hormones (e.g. testosterone) and its high content of omega-3 fatty acids has led to initial research on its potential role in the prevention of prostate cancer (Demark-Wahnefried et al. 2001). A current phase II study is investigating the use of a low-fat diet and/or flaxseed in preventing prostate cancer.

While these and other botanical agents show promise for a number of biological mechanisms related to cancer prevention, well-designed randomized controlled trials are needed in humans before they can be advocated as effective chemoprevention agents.

## 7.3 The Mind–Body Connection

Mind–body approaches to health are those practices that generate states of mental and physical relaxation (e.g. meditation), or those that strive to improve attitudes and emotions regarding health (e.g. psychotherapy and support groups). A long history of research has explicated numerous ways in which psychology interacts with physiology. Much of this work has been in the area of stress. The mechanisms of stress, and stress reduction through mind–body techniques, have been thoroughly described through more than five decades of research. Early work on stress and health focused on the correlates of risk and incidence

of heart disease (Friedman and Rosenman 1974). In the 1980s, the emerging field of psychoneuroimmunology demonstrated the relationship between the hypothalamus, psychological states and immune function. This work began to extend the paradigm of the stress response to incorporate much more sophisticated models of the interactions between mind and body (Ader et al. 1991; Pert 1997; Zorrilla et al. 2001).

Much of the CAM literature written for cancer patients provides thorough reviews of mind–body options to consider and suggests the potential use of mind–body techniques for prevention of recurrence of disease (Lerner 1994; Gordon and Curtin 2000). Theories and research exploring the relationship between immune, nervous, and endocrine systems relate these factors directly to the development and progression of cancer. Current theories and ongoing research are contributing to an understanding of the effects of analogues of hypothalamic hormones on hormone-dependent cancer, the role of opioids and melatonin on cytokines, gene modulation, and the immune responses to behavioral therapy in cancer patients (Temoshok 1987; Holland 1989; Schipper et al. 1995; Conti 2000; Elenkov and Chrousos 2002).

### 7.3.1 Coping and Immunity

Stress has been defined as virtually any experience activating the sympathetic nervous system (SNS) with its concomitant release of epinephrine and norepinephrine, rise in blood pressure and heart rate, and outpouring of other mediators to fight, flee, or freeze. More recent proposals have been made to eliminate the term stress as a mere reification and to speak about Hypothalamic-Pituitary-Adrenal (HPA) activation and sympathetic nervous system arousal. What leads to this activation and arousal is highly individual and cultural, arising from participation in a social environment in which positive and negative experiences are defined and value is placed upon aspects of life. Certain judgments or valuations of one's own life or life experiences leads to activation of the HPA axis, exerting effects on peripheral target tissues through glucocorticoids. This activation helps the individual survive a challenge by mobilizing fuels for energy, inhibiting reproductive behavior and increasing arousal and vigilance. Excessively long or frequent HPA activation and SNS arousal prevents recovery or adaptation, which leads to an increased risk of disease (Raison and Miller 2001). Dysregulation of the SNS/HPA axis (due to increased exposure to cortisol), is hypothesized to contribute to the physiological decrements that accompany diseases associated with aging and suppressed immune function, factors implicated in cancer risk.

Our social environments impact our bodies through relationships. For example, Kiecolt-Glaser and colleagues (2005) found that experimentally induced blister wounds healed slower after marital conflict than after supportive social interaction. Cytokine production in the area of the wound was also lower for interleukin-1-beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and IL-6. High-hostility couples healed at 60% the rate of low-hostility couples. High-hostility couples also produced higher levels of IL-6 and TNF-α the morning after a marital conflict than after a supportive social interaction when compared to low-hostility couples. More frequent and more intense production of pro-inflammatory cytokines could lead to increased susceptibility to a variety of disease states, including cancer.

Excess activation of the HPA axis or excessive sympathetic arousal can be reduced by interventions that combine meditation, breathing, and movement. Mind–body methods that emphasize breathing along with progressive muscle relaxation, meditation, yoga postures, or autogenic training (a technique that uses a combination of attention and somatic suggestion to assist relaxation) have been shown to reduce blood pressure among people with hypertension (Astin et al. 2003), to increase phagocytic activity (Peavey et al. 1985) and to lower cortisol levels (Schmidt et al. 1997; Kamei et al. 2000; Gaab et al. 2003). Mindfulness meditation has become one of the more standardized and popular methods of meditation taught in the West. Studies show direct improvements of immune function among other health indicators in response to this form of meditation, which focuses on breathing, relaxation and a full consciousness of one's surroundings (Kabat-Zinn 1994; Williams et al. 2001; Davidson et al. 2003). Popular among cancer patients since the late 1970s are the visualization techniques promoted by Carl Simonton (Simonton et al. 1978). Preliminary studies of this technique have shown improvements in leukocyte and NK cell activity in response to imagery of healthy immune systems and retreating cancer cells (Achterberg and Lawlis 1984; Kiecolt-Glaser et al. 1985; Gruber et al. 1988).

Qigong and Tai Chi exercises, ancient TCM practices that include breathing and movement, are often seen as mind–body approaches to health (Kerr 2002). Tai Chi is one of the forms of Qigong exercise that has become increasingly popular in the US; Qigong is gaining in use and popularity. The effects of Qigong and Tai Chi are clearly linked to the reduction of stress, including subjective assessment of anxiety and stress reduction, improvements in sleep quality; and more objective measures of reductions in blood pressure and sympathetic activity and increased parasympathetic activity (Audette et al. 2006; Channer et al. 1996; Cheung et al. 2005; Lee et al. 2003; Li et al. 2004; Skoglund and Jansson 2007). These practices hold potential for affecting the immune system; there are reports of increased phagocytic activity, lymphocyte transformation, and improved cytokine profiles among those who practice Qigong (Ryu et al. 1995; Chen and Yeung 2002) and similar results have been shown for Tai Chi practice (Sun et al. 1989; Wang et al. 2004). One of the few studies of Qigong exercise effects on immunological function published in the US showed a reduction in cortisol and an increase in the number of the cytokine-secreting cells (in particular, significant increases in the IFNgamma:IL-10 ratio) (Jones 2001). Although much of the research on the mind–body connection has been done in the context of cancer patients, immune system responses may arguably translate to cancer prevention for those who utilize mind–body techniques in the context of wellness (Engelking 1994).

### 7.3.2 Psychological States, Interventions, and Cancer

Although theories have been proposed to associate certain predispositions of personality or psychological factors with the development of cancer, the research has been inconsistent making it difficult to summarize conclusions (Garssen and Goodkin 1999). Prospective studies have implicated a number of possible psychological risk factors for developing cancer, with the most consistent finding being repression of emotion or depression (Dattore et al. 1980; Temoshok et al. 1985; Hislop et al. 1987; Temoshok 1987; Kaasa et al.

1989) yet even these have been disputed (Hahn and Petitti 1988; Kreitler et al. 1993). A preliminary understanding of these issues might best be gained in the context of survival, in that the factors influencing survival time may represent similar mechanisms that prevent recurrence.

Work with animal models has clearly shown a relationship between psychological determinants and tumor growth (LaBarba 1970; Sklar and Anisman 1981; Visintainer et al. 1983), but in the human arena the relationship between psychology and the course of cancer initiation and progression is less clear. Factors shown to improve outcomes include social support (Ell et al. 1989; Weihs et al. 2005; Waxler-Morrison et al. 1991), greater expression of distress, smaller numbers of severe or difficult life events (Ramirez et al. 1989), and "fighting spirit" (Pettingale et al. 1985). A discussion of the potential role of psychological variables moved to the forefront in 1989 with the publication of research that found a positive effect of a psychosocial intervention on breast cancer patients' survival time (a study that has since been called into question due to differences in baseline characteristics of patients and other study design issues) (Spiegel et al. 1989). This study kicked off a series of similar research projects, but all trials specifically designed to test the impact of a psychological intervention on survival have so far given inconclusive results, with some researchers concluding that the lack of significant findings demonstrates that such interventions simply do not impact survival time (Kissane 2007). Nevertheless, clinical experience points to the existence of a minority of cancer patients who make strong efforts to help themselves psychologically and appear to live longer than expected (Ikemi et al. 1975; Achterberg et al. 1977; Roud 1986; Berland 1995).

Cunningham and colleagues (Cunningham et al. 2000, 2002) have conducted exploratory research with metastatic cancer patients enrolled in a year-long psychospiritual intervention. Standard psychometric instruments did not predict length of survival in this study, but a subsequent analysis of qualitative interview data from 22 participants suggested that there may be a survival benefit among those who: (a) believed that the group activity would help them; (b) invested a greater level of effort in the group activities; (c) engaged to a greater degree in personal and spiritual change; and (d) had improved perceptions of quality of life as compared to those who did not.

The inconclusive results of previous randomized, controlled trials of psychological and spiritual interventions may be explained by patients who benefit, finding a way to get their needs met regardless of the randomization protocol. Mehl-Madrona and colleagues (2007b) found this effect in a study of asthma and CAM. Motivated patients optimized their exposure to CAM modalities regardless of the group to which they were randomized, but they did not voluntarily disclose this information to researchers. Those predisposed to involve themselves in psychological work may find opportunities in the context of an intervention or may seek such opportunities on their own. Researchers are likely naïve in thinking they can control patient behaviors to the extent needed for this type of research. Furthermore, if only a proportion of patients in an intervention become strongly involved in trying to help themselves psychologically, and theirs are the only lives substantially prolonged, this effect may be "diluted out" when group means are calculated. Another challenge to studies examining these effects includes the lack of inclusion criteria selecting patients for some degree of psychosocial distress. A randomized, controlled trial would thus need to be exceedingly large to produce a reliable treatment effect and would likely need to control for patients' expectations, level of involvement in the intervention or outside options, and

level of distress or need prior to study initiation. Study designs could be improved by exploring unique selection criteria for baseline predisposition of seeking "growth" experiences, selection of baseline distress and better choices of the factors assessed as potential mediators (Classen et al. 2007; Cunningham et al. 2002). Mehl-Madrona (2007) has argued that randomized, controlled trials of psychological interventions are not the best vehicle to demonstrate psychospiritual healing, given the many factors involved that cannot be controlled through randomization.

Given Cunningham's findings (Cunningham et al. 2002), there is reason to suspect that, although more challenging to design than originally thought, there is great potential for finding more robust relationships between the psychological, social, and even spiritual factors associated with patient involvement in their own healing. Preventing cancer, or preventing a recurrence of cancer, may similarly be dependent upon a set of psychoneuroimmunological factors more clearly demonstrated through studies that assess these relationships directly rather than through attempts at manipulating psychological and spiritual states indirectly through interventions. Although developing a sense of purpose in life, having a supportive community, and having faith for healing may not manifest in the same way with healthy individuals as with those who have been challenged by a diagnosis of cancer, these factors are worth examining to reduce risk of disease as well as improving patient quality of life. Continuing to explore social, psychological, and spiritual factors related to the mind–body connection may prove fruitful, but there remains a need for evidence regarding the specific mechanisms of these mind–body approaches that affect cancer outcomes.

### 7.3.3 Wellness

Much of the interest in CAM from the public and practitioners has been guided by and intrinsically linked to emerging views based on the concept of "wellness." The wellness concept became popular in the early 1970s, and out of that original thrust, many wellness centers began to emerge. The term "wellness" has come to denote a way of thinking about health that goes beyond the simple absence of disease. In theory, wellness is not necessarily sought from a position of avoiding disease, but is holistic in the sense that it seeks to achieve greater balance and awareness in body, mind, and spirit, an evolution of one's whole being (Benson and Stuart 1993).

Several of the culturally-based systems of health, such as TCM, Ayurvedic medicine, and practices associated with Native American healing, match the holistic precepts of wellness. Although there may be practitioners and patients with a single-minded focus on a particular modality, most proponents of CAM view health as multi-faceted, promoting balance and wellness of body, mind, and spirit. Because the systems promoting wellness tend to be broad in focus, prevention of a specific disease such as cancer is not an exclusive goal. Nevertheless, the expected outcomes of improved wellness include enhanced immune function, stamina and overall well-being, factors that may reduce the risk of cancer as well as other diseases.

There is a particular challenge for drawing conclusions about the cancer preventive potential of wellness-focused systems. Most are promoted as an entire wellness package, where a set of principles guide lifestyle, such as diet, form of exercise, meditation or other

mindful practices, spiritual views, and even botanicals, each specifically chosen for balance based on the philosophy of that system. In the context of cancer treatment, several of these systems propose paradigms of etiology, disease progression, and healing that are very different from the Western medical model, which focuses on the treatment of a specific diagnosis or symptom.

Researching these non-Western systems is problematic. The multiple modalities usually prescribed for treatment are based on individual readings of the various levels of involvement in body, mind, social, environmental, and spiritual factors. Research methods are not designed to test non-Western systems that treat every individual differently according to particular needs. Because of the lack of accepted research methodology that is able to test this holistic approach to health, it is important that health care practitioners become aware of, and researchers to begin to solve the puzzles of, scientifically understanding these integrated systems (Elder et al. 2006).

CAM practitioners promote balance for healing or for overall well-being, but the goals of achieving wellness are put into the hands of the individual taking responsibility for her or his own health (Wong and McKeen 2001). The movement toward self-responsibility in health and examining life as a whole are principles that sprouted in Western culture. This movement took place concurrently with a rekindled interest in more natural forms of healing that included mind–body approaches, spirituality, and non-Western medical practices, and has been especially powerful among cancer patients (Tatsumura et al. 2003).

An exemplar of this philosophy is the Wellness Community, an international network of non-profit centers "dedicated to providing free emotional support, education and hope for people with cancer and their loved ones" (Benjamin 1995; Penson et al. 2004). Using a whole-person (holistic) approach, patients are encouraged to actively support each other "to regain control, reduce feelings of isolation and restore hope-regardless of the stage of disease" (Penson et al. 2004). Varieties of meditation, Qigong, massage, yoga, Reiki, healing touch, and other such practices are commonly offered in these centers, making available to cancer patients a supportive community as well as opportunities for spiritual, emotional and physical healing.

Popular writers such as Dr. Bernie Seigel (Seigel 1990) and Dr. Andrew Weil (Weil 1995) have intrigued readers with stories of healing and profoundly changed lives. Many of these stories are recounted in books that catch the attention of the public, inspiring faith in the human ability to get well and stay well. For example, Dr. Caroline Myss, in her book about power and healing, suggests exploring symbolic meaning and balance, taking the reader through religious symbols, archetypal challenges, and stage-of-life passages to address life lessons and points of transition (Myss 1996). Moving through these lessons with conscious response is theorized to bring spiritual health cascading down through the psychological and physical systems. Even though Dr. Myss inspires the reader with histories of patients with cancer or pre-cancerous conditions who get well, there is neither sufficient documentation of measurable physiological outcomes over time nor comparison to matched cases to draw conclusions.

Research on spontaneous remission was more prevalent in the early 1900s; numerous examples defy our current understanding of the disease process (O'Regan and Hirshberg 1993). A common ingredient in these healing testimonies is spirituality (Hammerschlag 1988; Struve 2002). It is important for the reader to understand that these are testimonies, not scientific research. The role of spirituality in wellness, although only emerging in western

societies over the past few decades, might be seen as a reclamation of ways of viewing health and life that have been prevalent in most cultures and societies worldwide.

## 7.4 Indigenous Cultural Systems of Healing

Many cultures throughout the world have produced comprehensive, sophisticated systems of describing disease states and methods of healing that are very different from the Western medical model. What characterizes many of the systems, and distinguishes them from western medicine, is the tendency to consider health to be a system of balance among body, mind and spirit-often including social and environmental factors as part of the whole in some systems, rather than a constellation of biochemical and hormonal balances identified at a microbiological level (Villoldo and Krippner 1987; Achterberg 1977; Metzger 2004). Illness results from disrupting the natural order and disrupting harmonious relationships of all kinds. In these views, illness is not just something that happens to us or enters from without, but emerges from loss of personal power and balance that leaves us vulnerable to intrusion (Achterberg 1977). From African healing arts to Samoan fof from Native American ceremony to TCM, it appears that nearly all of the indigenous cultural systems view human health and prevention of disease in the context of the natural world around us as well as the interaction of physical elements with mind and spirit (Kaptchuk 1983; Mehl-Madrona and Dossey 2003; Mishra et al. 2003). Understanding these other view points or stories about the world is not just quaint anthropogy; it gives us the possibility to take a fresh look at our own world views and stories from outside eyes through which we can see our blind spots and unchallenged assumptions.

For example, within these world views, the soul may be seen as deliberately choosing illness to develop specific qualities and contribute to the community (this must be distinguished from certain New Age stories in which the conscious individual is attributed causality for cancer). Hillman (1996) reflected upon traditional spiritual healing within multiple cultures, observing that some cultures value symptoms and illnesses differently from Western culture, seeing them as not necessarily negative, but potentially useful for revealing a person's unique calling or destiny. They may emerge from a combination of accidental happenings, neither good nor bad, that coalesce into something that wants to be seen or heard. Indigenous cultures also speak of the life force (ki or chi in Asia) not flowing naturally, and illness as a signal that balance in the life force is needed. Symptoms, then, may be seen as part of an initial shamanic calling or a developmental opportunity, whose refusal can result in increasingly worse physical condition and even death (Walsh 1991).

Three classic and common concepts across cultures are those of power loss, soul loss, and spiritual intrusions (Horrigan 2003). Spiritist healers around the world believe in the idea of spiritual intrusion in which a person has an opening or void in the body and something comes to fill it. One could readily relate this to the contemporary psychological construct of resiliency. Cancer can also be seen as emerging from spirit possession, especially if one has already experienced significant soul loss (Horrigan 2003; Villoldo and Krippner, 1987). Villoldo and Krippner's informants told them that the entering spirit may trigger

in the host person similar symptoms to what the entering spirit suffered before or during death. In this view, entire families may be affected by these spirits, but frequently only the most sensitive person (translatable as the "least resilient"), often the youngest or the oldest, may be affected. Concepts such as intrusive spirits form a kind of plot line that can readily be translated into modern terms, like the unwanted intrusion of anger and hatred or bitterness into a person, who becomes unable to clear those emotions.

Each of these systems of healing typically has theories of how particular diseases develop, and consequently suggest tactics for prevention. Health and disease is understood in the context of a particular person's experience of life, family, dietary and environmental factors, social conditions, and spiritual responses. Ayurvedic medicine and Native American healing are given as two examples of systems of healing that promote living in harmony with nature, with one's given constitution, and that promote connecting to spirit as ways to stay healthy.

### 7.4.1 Ayurveda

In Ayurvedic medicine, the build-up of toxic residues termed "ama" (more than physical substances in biomedical terms, but more subtle essences produced by imbalances in living, diet, and mental/emotional states) is believed to be one of the main causes of cancer. Recognizing one's primary "doshas," or essential natures, and living a lifestyle to manage and balance these elements alongside cleansing techniques designed to rid the body of ama, are considered to be the best approach to avoiding cancer from the Ayurvedic point of view (Chopra 2000; Herron and Fagan 2002). Ayurvedic medicine uses the terms "mind" and "consciousness" to refer to the origin of physical conditions. "When you look at Ayurveda's anatomical charts, you don't see the familiar organs pictured in Gray's Anatomy, but a hidden diagram of where the mind is flowing as it creates the body. This flow is what Ayurveda treats" (Chopra 1989).

Although the combination of these many lifestyle and mind–body approaches are seen as central to treating cancer, research has focused primarily on Ayurvedic herbal formulas in more recent years. For example, *Maharishi Amrit Kalash,* an Ayurvedic food supplement, has been shown to regress mammary tumors, inhibit liver carcinogenesis in rats and has been evaluated for other anti-cancer effects due to its potent anti-oxidant properties (Arulkumaran et al. 2007; Fields et al. 1990; Sharma et al. 1990). Other Ayurvedic combination herbal preparations that have been tested include *Kalpaamruthaa,* demonstrating inhibition of carcinogenesis in animal models (Penza et al. 2007), and *Ashwaganda* (derived from the plant, Withania somnifera Dunal), found to have selective anti-tumor properties through activating growth arrest and apoptosis in mice and in vitro growth assays of normal and human transformed cells (Garodia et al. 2007; Widodo et al. 2007).

Given that these preparations are only one aspect of the many modalities used in Ayurvedic medicine and that more emphasis is placed on building balance through assessment and lifestyle changes to support essential constitutions, much work must be done to test the potential for this system to affect cancer risk. Singh (2002) provides a much needed framework for presenting various aspects of Ayurvedic practice in cancer treatment, linking theoretical constructs of that system to potential explanations of medical biomarkers that

could be explored to begin explicating mechanisms of action for treatment and prevention. Such a framework would provide methodical guidance for testing this as whole system approach (Elder et al. 2006).

Deepak Chopra, M.D., has done much to promote the concept of mind–body healing and wellness through his center and in his book entitled *Perfect Health; The Complete Mind–Body Guide* (Chopra 2000). This book repackaged Ayurvedic principles of healing into an approach more palatable to Western tastes. The details of the Ayurvedic system are generalized to principles that are recognizable in modern wellness teaching. Such programs continue to represent the basic tenets of wellness – balance in life and responsiveness to life's lessons. These basic tenets are hypothesized to bring optimum health, and hence are also purported to help prevent cancer.

### 7.4.2 Native American Healing Traditions

With so many tribes indigenous to the Americas, each with unique healing traditions, it becomes impossible to define a particular tradition as representative of Native American healing. There are, however, some themes that are common throughout the Americas. For example, Native American healers generally do not conceptualize a medicine-religion differentiation. Healing must acknowledge an Inner Healer. According to Dineh (Navajo) healer, Thomas Largewhiskers, this inner force explains why people get well or not, or how they stay healthy in the first place (Largewhiskers 2004). Many tribes and other indigenous cultures teach that spirit is indivisible from mind and body and that a healthy balance and connection with spirit is the path to prevention of disease.

An important source for connecting with spirit for healing and balance, for healthy and ill alike, is ceremony. Ceremony is not unique to Native American culture; it is often represented in one form or another in health systems that include a spiritual component (Hammerschlag and Silverman 1997). The importance of ceremony is consistent with the hypothesis that religiosity/spirituality may be associated with physiological processes, including cardiovascular, neuroendocrine, and immune function (Seeman et al. 2003). Immune function is important in cancer and its prevention. Although the evidence has not demonstrated that spirituality slows the progression of cancer, studies conducted to date have been small and limited (Powell et al. 2003). In cultural systems that involve ceremony, prevention should address mind, body, and spirit.

Native American healing practices hinge less upon the skills of the healer and are placed more in the hands of the spirits or the Creator who respond to the collective requests of the community and "doctor" the patient. Success is seen as outside the realm of mere humans. Nor does the healer take credit for improvements or even cures, preferring to remember the spirit helpers and the Creator. Healers build their reputation based upon the power of the spirits who assist them. Healers and other participants in ceremonies "see" or listen to messages provided by spirits to help the patient, including how to change aspects of the person's individual spirit or energy body through sound, touch, or prayer. This is a traditional way of viewing what is now often called "energy medicine," a field that combines technology, physics, and ancient wisdom for healing.

7

### 7.4.3 Energy Medicine

Energy medicine is often used to refer to practices that involve subtle or very low intensity non-material stimuli for purposes of healing (Rubik 1995). These stimuli may be: (a) artificially generated fields, including measurable vibrations such as sound, visible light, laser beams and electromagnetic fields [e.g. pulsed electromagnetic field (PEFM) therapy]; or (b) healing energies generated by and purportedly transmitted to humans (e.g. Reiki, Healing Touch) or intentfully balanced from within through various meditative movement practices (e.g. Qigong exercise, Yoga, Sign Chi Do). These stimuli are theorized to work by causing a change in the human biomagnetic energy field and subsequently in biochemical responses.

Energy medicine includes a wide range of therapies. As noted above, many of the indigenous systems of healing acknowledge a life force such as Qi (the force that runs through meridians as defined in TCM), prana (in yoga practice and Ayruvedic medicine) or the fields of energy perceived and manipulated by those practicing any of the forms of energy healing. For purposes of this review, a broad definition will be used to encompass any modality designed to improve overall balance or improvement in some aspect of the human bioenergy field either through internal intent or external application. The mechanisms by which energy healing may work to improve health are still being explicated. Without knowing yet whether there is one underlying principle or many ways in which non-material stimuli might affect energy fields and, in turn, biological outcomes, these modalities will be considered as similar in this review.

The purpose for addressing the emerging field of energy medicine in the context of cancer prevention is twofold: (1) to explicate the non-Western theories underlying energy medicine approaches; and (2) to discuss the theory inherent in energy medicine-based systems that early correction of stagnation or imbalances in energy patterns may lead to the prevention of cancer. Many non-Western systems of healing are based on the concept that there are flows of energy or essence patterns in the body that are dynamically balanced and must not be blocked; the blockages of energy allow the development of disease, including pre-neoplastic lesions and eventually cancer. Energy-based medicine systems attempt to unblock these flows through various methods such as acupuncture treatments, specifically designed Qigong exercises, or externally applied biomagnetic emissions from the hands of a healer. Even more remarkable is the hypothesis that detection of early neoplastic changes may be possible with methods that assess Qi imbalance.

The term “biomagnetic field” refers to the various electromagnetic fields that run through and around the body (Oschmann and Pert 2000). Electrical impulses generated in the heart and brain (as measured conventionally via electroencephalograph, or EEG, and electrocardiogram) are known to generate biomagnetic fields. Such fields have been detected by trained energy medicine practitioners as emanating from the whole body as well as specific parts, notably the hands.

The most controversial aspect of energy medicine is the assumption that biomagnetic fields are more than artifacts of electrical activity in the body and that they not only provide indicators of health, but that they can be altered to produce improved states of health. For example, TCM is based on the concept that there are flows of energy that can be mapped representationally across the surface of the skin (although they are believed to

flow through and beyond the limits of the physical body). These flows have names that link them to organs of the body (e.g. liver meridian or kidney flow), but their function is seen as much broader, including emotional and symbolic as well as physiological functions.

A more familiar and widely accepted version of energy medicine comes in the form of devices designed to deliver various stimuli. Applications of electromagnetic fields within certain frequency ranges have been shown to have a number of biological consequences with potential application in medicine. Pain control using transcutaneous electrical nerve stimulation (TENS) is one of the more well-known developments in energy medicine. Tissue regeneration, bone repair (Sharrard 1990), improvements in osteoarthritis (Trock et al. 1993), soft tissue wound healing (Becker 1990; O'Connor et al. 1990), neuroendocrine modulations, and immune system stimulation (Cadossi et al. 1988a, 1988b; Cossarizza et al. 1989) have all been documented in response to various forms of electromagnetic stimuli. PEMF therapies used in many of these established applications have shown that certain body tissues are particularly responsive to specific frequency ranges (e.g. 7 Hz for bone growth, 15–20 Hz for capillary formation and fibroblast proliferation, and 2 Hz for nerve regeneration) (Sisken and Walker, 1995). The usefulness for such externally, artificially applied therapies have not been demonstrated for cancer prevention directly; but recent studies show that selected frequencies of PEMF therapies inhibit tumor growth in animal models (Salvatore and Markov 2004) and can inhibit tumor angiogenesis (Markov et al. 2004). Even with the recent proliferation of research on PEMF therapies and potential health effects, there is still a limited understanding of how these effects occur and what specific dimensions of PEMF therapies (e.g. frequency, amplitude, waveform) are required to match targeted tissues and intended effects (Markov 2007).

There are numerous modalities of healing that are theorized to involve the interaction of biomagnetic fields between two humans, one "sending" healing and the other "receiving." These include practices such as Reiki therapy, Johrei, Healing Touch, Therapeutic Touch, and laying on of hands in the context of prayer (Anderson 2001). Although practitioners from these various fields of practice may argue for marked differences in their intent and work, studies examining the biomagnetic fields emitted from the hands of healing practitioners across disciplines found similar emissions. Pulsing electromagnetic fields of extremely low frequency (ELF) emitted from the hands of healers have been detected, the majority falling between 7 and 10 Hz with a range of 0.03–30 Hz (Zimmerman 1990; Seto et al. 1992). Similarly, healing practitioners using a variety of modalities also demonstrate similar EEG signatures while in the healing mode, altered state, or in prayerful focus (Beck 1986). Although healing effects of emissions in these studies were not tested, the frequencies measured all fell into the same range as electromagnetic device therapies shown to have specific healing outcomes (e.g. 7–10 Hz) (Sisken and Walker 1995).

Other than the similarity of wave frequency between the PEMF therapies of targeted devices and the range of PEMF therapies from the hands of healers, there is little known about either measurable or putative energies or mechanisms of effects. Lutgendorf (2003) notes that research on energy healing has been confined mostly to studies of cancer cells in vitro, with positive findings generally providing evidence with well-characterized molecular endpoints, but that there have also been multiple studies with negative or equivocal findings. For example, studies of emissions from the hands of skilled healers have demonstrated cytotoxic effects, as tested on liver, breast and pancreatic cancer and glioma cells

(Ohnishi et al. 2005, Yan et al. 2005) and no effect on fibroblasts and human umbilical vein endothelial cells, while other studies have not demonstrated significant effects of human-delivered energy healing on cancer cells (Yount et al. 2004a; Yount et al. 2004b; Zachariae et al. 2005).

One of the oldest documented forms of energy healing is the practice of Qigong, central to the system of TCM. Foreign to Western medicine and philosophy, Qigong is typically considered to be comprised of two forms, external and internal Qigong. The potential for Qigong exercises (i.e., internal Qigong) designed to balance the meridians to have an effect on health indicators such as cytokine profiles has already been discussed in the section on mind–body interventions. External Qigong or external qi healing ("wai qi" or "wei qi") (Cohen 1997; Johnson 2000), an ancient Chinese method of healing touch, refers to the emission or conscious application of qi. It is administered by transfer from the hands of experienced Qigong practitioners to other individuals for the purposes of healing or improving health. Emitted qi also has been shown in numerous studies to have immune modulating effects, mostly reported from research conducted in China with incomplete information on study design (Liu 2004). Preliminary evidence is good for demonstrating potential of qi emission to strengthen cellular immune function in animal models and humans.

More notable are the results that are beginning to be published in the US on effects of qi emission (external Qigong) on suppression of growth of induced lymphoma in mice. Although results were inconclusive relative to varying abilities of practitioners, one of the studies showed significant differences in tumor inhibition between intervention and control mice (Chen et al. 2002).

Beyond these claims, most of which focus either on healing for specific conditions or improvement in immune status, there is much to be learned from the theories and practice of Qigong. In this paradigm of health, the goal of balancing qi is woven through the practices. From the qi emission from the hands of a master practitioner who "sees" the blockages and areas of need in the biofield, to the exercises designed for movement of qi through specific meridians, to the herbal remedies that stimulate, calm or unblock meridian flow, each modality is intended to move one toward greater health. Validating through research a system so focused on prevention of disease, catching the imbalances in the energy field before illness manifests, seems unlikely.

Many people are familiar with the idea that TCM includes a range of modalities, such as acupuncture and herbal remedies. What is less well-understood is that these treatments are all designed to influence the energy map of the body; in TCM, a network of energy lines called meridians represent various organs and functions, physical and emotional, and the balance of these are purported to maintain health. The theory behind a particular remedy is not necessarily direct action upon an organ or a biochemical reaction (as is the case with Western views of herbs), but rather its effects on promoting, inhibiting, or balancing the targeted energy meridians (Kaptchuk 2002).

Explanations for what structures in the body might account for positional relationships to energy phenomena have more recently been proposed, most notably in the context of acupuncture. The clinical efficacy of acupuncture has been demonstrated for a variety of outcomes, yet the theories of how it functions according to TCM principles are often not accepted even by physicians who practice this form of energy medicine (Kaptchuk 2002). Neurohumoral approaches in acupuncture research were instrumental in establishing the scientific validity of acupuncture. Recent advances in the morphogenetic singularity theory

suggest that acupuncture points originate from the organizing centers in morphogenesis. Possible mechanisms of action are not necessarily dependent upon an association with nervous or lymphatic systems; acupuncture points and meridians have been shown to have high electric conductance that is related to high density of gap junctions in cellular structure (Shang 1993; Shang 2001).

There is evidence that acupuncture has immunomodulatory effects (Petti et al. 1998; Kaptchuk 2002). For example, T cells and activity of NK cells in cancer patients treated with acupuncture have been shown to remain stable over the course of chemotherapy (Ye et al. 2002). In a group of relatively healthy young volunteers, acupuncture normalized leukocytes (Mori et al. 2002).

Speculations about how energy healing interventions may work vary. One explanation is that "intent" may have an effect through the mind on the frequencies emitted and received. Recent developments in the science of mind and experiments of consciousness are beginning to reveal effects for "intent" on physical matter, such as re-configuration of water molecules (Tiller 1997). Another theory suggests that physical resonance develops via "entrainment" (an accepted principle in physics whereby pulsing of waves or rhythms produced by pendulums in close proximity will entrain to the point of resonance) between the healer and target. Evidence of the potential for this explanation is provided in the work of Russek and Schwartz, who show that even without intent for healing, two people sitting quietly with eyes closed in the same room will tend to attain coherence between both cardiac and brain wave rhythms (Russek and Schwartz 1994, 1996).

The nature of human biofield-based energy healing has not been fully explained, but the effects have been well documented in randomized, controlled clinical trials on healing interventions such as Healing Touch, Therapeutic Touch and similar modalities. Despite continuing questions about exact modes of action, energy healing has been shown to significantly increase secretory immunoglobulin A (Wardell and Engebretson 2001; Wilkinson et al. 2002), enhance immune system response (Quinn and Strelkauskas 1993; Ryu et al. 1995; Kataoka et al. 1997) and produce relaxation or stress-reduction effects in general (Wirth et al. 1997). Other research has shown consistent results for improving quality of life and reducing stress-related symptoms in cancer patients undergoing treatment (Post-White et al. 2003; Cook et al. 2004). Although the application of the findings from these studies and the potential for cancer prevention has not yet been investigated, the results of these studies should attract enough attention to begin investigations of effects on biomarkers related to cancer risk or protection.

Large, randomized clinical trials that also allow for individualized treatment and practice plans, as is common in many forms of CAM, do not currently fit the research design standards in the US. Until research is able to be designed to encompass such studies, only small parts of this puzzle may be examined to assess the potential of CAM. One such puzzle piece may be the research that is beginning to emerge in the field of energy medicine through instruments designed to assess meridian flow.

Although the use of diagnostic methods such as "seeing" imbalances in the human energy field (the mark of a Qigong master being the ability to see or detect qi imbalances without the need of questioning, blood tests, or even hands-on pulse readings) may seem foreign to the western medical mind, there are recent developments in measurement of biofield emissions that correlate with and validate these fields. For example, acupuncture

meridians and stimulation/collection points are detectable via instruments, such as the MSA-21, that assess electrical impedance along the meridians (Roberts et al. 2002).

Instruments are available to assess purported flow of energy through meridians, such as the Gas Discharge Visualization device (GDV). The GDV captures the biofield emissions surrounding each of the ten fingers resulting in the measurement of biophotonic discharge that is in direct proportion to the amount of energy flowing through the 14 main meridians. The coronal discharges measured by the GDV are detected as a result of the human bioenergy field interacting with the electrical field pulsed through the device. At the time of measurement, the emitted light from the interaction is captured by a video camera, and then the images are analyzed with sophisticated software using a mathematical tool known as fractal dimensionality. The data can be integrated over the whole body resulting in a quantifiable analysis of the energy flow and balance throughout the meridian system (Korotkov 2002).

Experiments with the GDV in Russia have suggested potential for the detection of breast cancer. A study of 194 women, 140 with breast cancer, was conducted to assess the diagnostic potential of the GDV to see how it may be used to reflect the patient's "energy state" in the process of chemotherapy, radiation therapy, and surgery. Statistically significant differences were found between the GDV parameters of the cancer patients when compared with a healthy control group. The patients with cancer had a wide range of stages and diagnoses (Korotkov 2002).

Despite growing evidence that biofields may be related to biological functions and may possibly precede material changes (Tiller et al. 2001), it will take more rigorous research to define ways that protecting from assaults or correcting imbalances in those fields may affect health. There are experiments going on everyday through exposures and behaviors, but we cannot fully understand the role of biofields in health until they are evaluated in randomized controlled trials.

Nevertheless, energy medicine practices are common throughout the world. In China, the largest population on earth practices Tai Chi daily to balance energy fields. Although no correlation has yet been established, cancer rates in China are generally low (except for cancers directly attributable to tobacco) (Yuan et al. 1996). In fact, reviewing cancer rates worldwide, one might want to consider moving to Qidong, China as a "best practice for preventing cancer." The role of culture, health practices and spirituality, and many more suspects in the theatre of life exposures, need refined exploration. But until our understanding of mechanisms of energetic influences on the body and the biofield is understood in terms of the dominant microbiological paradigm, the research will neither be noticed nor applied to cancer prevention.

## 7.5 Conclusions

CAM is a continuously developing field, challenging researchers to find new epistemologies to match complex systems and competing evidence. For example, despite a long history of holding certain hormones responsible for hormone-related cancers, new evidence is accumulating on the potential of endogenous and/or bioidentical supplemental progesterone to

have protective effects against several cancers (Cowan et al. 1981; Formby and Wiley 1998; Malet et al. 2000; Horita et al. 2001; Dai et al. 2002). As findings emerge from the cancer treatment realm, its potential application to cancer prevention may be better understood. Another potential source for exploring alternative prevention approaches is to observe population-level behaviors, not only where indigenous systems of healing are commonly practiced, but other factors that are not neatly tied to current medical models, nutrition, and conventional exercise. It may be important to find ways to investigate on a large scale what cultural lifestyles and accompanying methods of healing used in those cultures, may be associated with lower rates of cancer. In such explorations, it will be important to solve the problems of separating out cultural and genetic influences and ruling out spurious correlations from true effects. It may be necessary to develop an appropriate research paradigm to apply to trials on lifestyles before the potential for such systems is fully known.

In the meantime, smaller-scale studies may begin to uncover select mechanisms of action of the elements of CAM. One of the primary funding agencies for examining alternative approaches to cancer treatment, control, and prevention of recurrence, the US National Cancer Institute (NCI) Office of Cancer Complementary and Alternative Medicine (NCCAM), has shifted many modalities previously considered CAM to conventional status. In particular, NCCAM now suggests that many of the mind–body approaches for reducing stress and improving quality of life among patients with cancer or cancer survivors has become mainstream due to well documented theoretical foundation and scientific evidence (e.g. patient education, biofeedback, and cognitive-behavioral approaches). These mind–body approaches are now assigned to funding within the main body of NCI divisions unless the focus of research is more clearly placed on novel mechanisms of action for cancer that have yet to be investigated. Currently, both in the current NCI research projects and in the statements of the modalities suggested for investigation (e.g. phase I/II trials of CAM mechanisms of action), it is evident that in the future there will be a broader research evidence base upon which to evaluate CAM approaches. This is good news; as the consumer continues to adjust lifestyle, seek wellness and attempt to prevent disease through conventional as well as alternative systems, knowledge that comes from well-designed research may guide this search toward more effective prevention strategies.

The creative search for alternative modes of treatment and supportive care for cancer patients may serve as a useful starting point to identify potential candidate agents and practices for research in prevention. In this light, some of the therapies gaining popularity for supportive care may gain attention in the future as more than simply positive experiences. For example, music, art therapy, and guided imagery all have a long tradition of use with cancer patients, and many oncologists encourage their patients to integrate such extra-medical practices into their treatment program. Often these are not seen as modalities that could affect the course of cancer, only to help the patients cope psychologically with their disease. However, as more evidence accumulates that such activities, even for healthy patients, might affect overall health and immunity, potentially even at the cellular level (Chopra 2000), these may also be explored as ways to improve one's chances of avoiding cancer. Until that day, one should consider adding flaxseed and curcumin to a diet rich in fruits and vegetables, engage in some form of daily meditation, practice yoga, tai chi or Qigong to the point of obtaining a balanced GDV reading, and watch the research unfold over the next decade.

7

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# Telemedicine in Cancer Prevention

# 8

**A.M. Lopez, L. Kreykes**

## 8.1 What is Telemedicine?

Telemedicine is the use of telecommunications technology to provide health care services to patients who are geographically separated from a physician or other health care providers. Interest in using telemedicine to solve a number of problems in health care delivery has been growing worldwide in recent years. The types of teleconsultation services could include real-time and store-forward consultations, continuing medical education, and patient information sessions. (Lopez et al. 2005). The *Telemedicine Action Report* of the Western Governors' Association, published in 1994, outlined barriers to implementation of telemedicine and recommended a variety of actions and solutions including the initiation of statewide telemedicine networks (Cooley 1996).

Store-forward consultations are performed when the diagnostic question does not require direct audio and video interaction or a virtual physical examination. Teleradiology is the most common example of this type of teleconsultation. Patient information can be recorded as an image, either through video, photograph, or through a diagnostic test, such as a radiograph, and can be transmitted after the initial consultation with the patient and referring physician has taken place. The teleconsultant reviews these data at a later time and provides a diagnostic assessment and therapeutic recommendation to the referring practitioner on his or her findings. Although the collection of patient data and the assessment of patient data by the teleconsultant are disconnected, accurate diagnostic assessments can be made based on the data transmitted and high-levels of patient satisfaction have been reported (Lopez et al. 2005).

Real-time teleconsultations bring the patient and the teleconsultant virtually face-to-face. When medically necessary, a patient presenter may also be part of the clinical interaction. Ideally, the presenter is the referring clinician. As the person requesting the consultation,

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she or he is acutely aware of the nuances of the clinical request and is best able to present the patient's condition. Under certain clinical conditions, these interactions have provided the referring clinician/presenter with continuing medical education credit as they provided real-time, interactive educational experiences. Real-time interaction also allows for direct communication between the teleconsultant and the patient and are rated highly with regards to patient satisfaction (Lopez et al. 2005).

Telemedicine can increase access to quality, timely and cost-effective specialty healthcare. Telehealth technologies have the capacity not only to revolutionize the way physicians care for patients but also to change the way clinical care is structured with changes in workflow, quality management and transmission of patient data, factors which can influence the cost of care (Ricke and Bartelink 2000). Specifically, the costs of teleoncology practice have been assessed in multiple settings. In comparing a teleoncology clinic with a fly-in outreach clinic, both in rural areas with a traditional clinic in a city hospital, Doolittle, et al demonstrated the cost and clinical efficacy of the teleoncology practice (Doolittle et al. 1997). The authors built on this work and initiated discussion on the importance of developing methodologies to evaluate cost efficacy for telemedicine practices (Doolittle et al. 1998). Critical to cost efficacy assessment is appropriate use of technology and defining the appropriate technology for the intended diagnostic question. Although telemedicine technology can prove to be both clinically and cost effective in diagnosing disease, therapeutic costs can remain a barrier to care (Kvedar et al. 2006). As the field of telemedicine continues to grow, the benefits of telemedicine will continue to overcome the barriers to specialty healthcare experienced by many. As face-to-face medical care has begun to embrace prevention, so too, has telemedicine begun to encompass prevention, health promotion and wellness.

## 8.2 Telemedicine in Cancer Care

With the increased prevalence of a cancer diagnosis, more patients are identifying as healthy patients at risk of a future cancer. These patients seek cancer prevention services but can find formidable geographic barriers. Although cancer prevention services may be provided by primary care providers, specialized genetic and high risk follow-up and prevention is most often provided in cancer centers and by cancer prevention specialists. Onega et al studied access to oncology care in the United States and found travel times of 1 h or greater to National Cancer Institute (NCI) designated cancer centers for the majority of citizens with greater travel times not only to NCI designated cancer centers but to any cancer care for native and nonurban populations (Onega et al. 2008). It is estimated that fully 25% of the population in the United States lives in remote or rural areas (Hightower 2003). Limited access to care can result in patients making choices about care based not on best medical practice but simply in terms of availability. With the anticipated workforce shortages in health care, ways to extend and improve access such as via telemedicine, will be significant assets that will ameliorate health outcomes (Onega et al. 2008).

Telemedicine applications in cancer care have been myriad. The technology has increased access to specialty consultation, to multidisciplinary care, to cancer clinical trials,

to supportive and adjunctive care, including home health care, to educational programming for patients, mid-level providers and physicians in primary and specialty care, including interdisciplinary tumor boards (Doolittle and Allen 1997; Kunkler et al. 1998; Martino et al. 2003). Although overall well-accepted by both clinicians and patients, occasional technological barriers persist and business models have not been well-defined (Olver and Selva-Nayagam 2000).

Patient satisfaction with the clinical experience does not differ significantly between in-person oncology and the teleoncology visit (Weinerman et al. 2005). Considering the outreach efforts of many programs and oncology practices, it is likely that the institution of teleoncology practices will result in cost-savings, clinical efficiencies and high levels of satisfaction and acceptance among both patients and clinicians (Allen et al. 1995).

## 8.3 Primary Cancer Prevention

Health promotion technologies lend themselves well to prevention education. Direct videoconferencing methodology can be used to bring the intervention support closer to the patient. Although some of this work was initiated with a tape of information or instruction, the technology, whether phone or videophone, can allow for direct real-time interaction with study personnel to effect behavior change.

### 8.3.1 Smoking Prevention

A randomized controlled smoking cessation telehealth study tested the efficacy of a lifestyle intervention in two populations. Each group needed either primary or secondary prevention efforts to reduce cardiovascular disease risk among mid-life individuals. Results demonstrated clinical efficacy of the primary prevention intervention. Follow-up evaluation at 1 year also confirmed efficacy (Wister et al. 2007).

### 8.3.2 Nutrition Counseling

A baseline survey was conducted at Bassett Healthcare on outpatients who had follow-up appointments with subspecialty health professionals. The survey revealed that nutrition counseling has the highest no-show rate and largest percentage of patients who do not reschedule their appointments. This program's goal was to determine if appointments for nutrition counseling would increase once the barriers of distance, transportation, and time were removed through the use of telemedicine. To achieve this goal, the Clinical Nutrition Department is utilizing interactive videoconferencing equipment (e.g. PictureTel Group System) to provide outpatient nutrition counseling. This system

enables the Registered Dietitian to observe and communicate with the patient at the outreach clinic despite the miles separating them. Six Bassett Healthcare outreach clinics are currently participating in this project. Primary care providers at each clinic refer patients for telemedicine nutrition counseling. Pertinent medical history is sent to the Registered Dietitian for review prior to the patients' appointment. Support staff assists in patient scheduling, data collection, and equipment function. A phone survey and patient questionnaire are being used to assess patient satisfaction and compliance. To date, many positive outcomes have resulted from this project, including: (1) the number of appointments has increased; (2) no-show and cancellation rates have decreased; and (3) follow-up appointments have increased. Surveys indicate a high degree of satisfaction with telemedicine nutrition counseling. In addition, patients have realized a saving in time and travel expenses. This program has identified the need for interactive video teleconferencing in a rural population and has demonstrated the benefits of providing this service (Johnson et al. 2001).

### 8.3.3 Exercise Education

Although physical activity is increasingly recognized as a modifiable risk factor for cancer prevention tele-exercise interventions have targeted cardiovascular health. These interventions have primarily targeted geographic barriers and have utilized the Internet as a medium for delivery of "virtual" exercise programs. Delivery of exercise education within our own program has utilized videophone technology to deliver Qigong exercises to patients who are awaiting heart transplantation. This experience was well accepted and appreciated since the patients were able to participate in the sessions even if they were unable to travel.

In a randomized controlled study on delivery of a cardiac rehabilitation program (CRP) to patients at a distance, the virtual CRP group significantly improved their HDL-C, triglycerides, total cholesterol:HDL-C ratio, and exercise capacity as assessed in metabolic equivalents, weekly physical activity, and exercise specific self-efficacy. No significant improvements were noted in the control group. The virtual CRP results were similar to historical controls in a standard CRP. Participant responses from exit interviews of the virtual CRP were unanimously positive. This latter finding may be most significant for long-term behavioral change, since high user acceptance may have implications not only for generalizability to other patients who do not have access to traditional hospital-based CRP but also for long-term use of the intervention (Zutz et al. 2007).

In post-stroke management, telerehabilitation has been successfully piloted in a single-arm study with videoconferencing technology. The intervention consisted of education and exercise and psychosocial support conducted by a physiotherapist via videoconference. After the intervention, there were significant improvements in The Berg Balance Scale, State Self-Esteem Scale, Medical Outcomes Study 36-item Short Form—all subscales and stroke knowledge. All subjects accepted videoconferencing well (Lai et al. 2004).

### 8.3.4 Genetic Counseling

Clinical consultations in genetics are not often available outside of a university or tertiary care center. Incidence of genetic problems in children is increasing and is of great public health concern. Current models for genetic health care delivery largely rely on specialist travel to communities in need. Unfortunately, this physical presence results in sporadic care that may leave the patient and family feeling unsupported for large periods of time. In an effort to provide a virtual presence in these communities, the Arizona Telemedicine Program has established a telegenetics outreach program. This program provides genetics services on an ongoing basis and thus supports continuity of care. This project built on existing relationships with rural communities and was enhanced by the mutual benefit achieved by this intervention. In an evaluation of the services, it was found that patients strongly agreed that they found telegenetics consultation to be beneficial, patients agreed that having the telemedicine visit now was preferable to waiting for a face-to-face appointment with the same doctor. In fact, overall, patients did not see a need to seek in-person genetic counseling services in the future as they were so satisfied with the telegenetics service (Cunniff et al. 2007).

There are few published studies regarding the use of telemedicine in counseling families with a history of cancer. Anecdotal experience would confirm that telegenetics consultations would lend themselves well to videoconferencing communication. Coelho and colleagues (Coelho et al. 2005) evaluated cancer genetic counseling when conducted via telemedicine and compared to face-to-face consultations. Participants were placed into a telemedicine group or a face-to-face group depending on their geographical location. Telemedicine consultations took place using real-time videoconferencing technology integrated services digital network (ISDN) digital telephone lines (specifically, ISDN6). Sixteen participants were evaluated in the telemedicine compared to 21 in the face-to-face group and all participants were asked to complete both a pre-counseling and post-counseling questionnaire, which assessed their understanding of cancer genetics, anxiety levels, satisfaction levels, and allowed for personal comments. In both the telemedicine and face-to-face groups, a significant reduction in cancer related anxiety levels and high satisfaction levels were reported. There was a trend towards increased cancer genetic knowledge post genetic counseling in both groups. The results show that telemedicine is a useful alternative by which to provide cancer genetic service (Coelho et al. 2005). Kaiser Permanente began to develop and implement practice guidelines for genetic counseling referrals for persons at inherited susceptibility for breast and ovarian cancer. Implementation of these guidelines and protocols required dissemination of the "high-risk guidelines for breast and ovarian cancer, dissemination of patient and physician educational materials on the breast cancer guidelines, monthly classes, taped health phone messages for patients, interactive videoconferencing for physicians, training seminars for medical geneticists, publication of articles on breast cancer and genetic risk in health plan member- and physician-directed magazines, identification and training of clinical specialists and supporting clinicians to care for patients before and after counseling, individual counseling and testing of patients and families, and development of a data registry" (Kutner 1999). The videoconferencing education is an extension of continuing education efforts throughout the country.

### 8.3.5 Psychosocial Support

Women with breast cancer in rural areas are likely to exhaust their usual sources of psychosocial support while still facing challenges posed by breast cancer, but are unlikely to have access to professionally led support groups. In a community-based project, the feasibility and acceptability of providing support groups to women with breast cancer in a large rural area using videoconferencing and a workbook journal was assessed (Collie et al. 2007). Additionally, this study assessed the intervention's potential to reduce distress, increase emotional expression, and to increase self-efficacy for coping with cancer. Twenty-seven women in the Intermountain Region of northeastern California participated in eight support group sessions led by an oncology social worker. All sessions were conducted via videoconferencing. The videoconference sessions were both feasible and acceptable to participants. Older as well as younger women were comfortable using videoconferencing. Participants reported that the groups were valuable because they promoted information sharing and emotional bonds with other women with breast cancer. The results emphasize the importance of a professional facilitator and note the advantages of using videoconferencing for support groups. Pretest and posttest comparisons showed significant decreases in depression and posttraumatic stress disorder symptoms. The results suggest that the intervention has the potential to provide a valuable service that is not readily available in rural communities (Collie et al. 2007). Structured phone support for breast cancer patients and their primary support person while undergoing adjuvant therapy in both urban and rural settings have also demonstrated efficacy (Badger et al. 2004; Badger et al. 2004b; Badger et al. 2005; Badger et al. 2007; Dorros et al. 2006; Segrin et al. 2006). Evaluation of this intervention in diverse populations is currently ongoing.

## 8.4 Secondary Prevention

### 8.4.1 Breast Cancer

Breast cancer is increasingly cited as the most common cancer diagnosis in women throughout the world. Women in the US have a one in seven lifetime risk of developing the disease. As the prevalence of the disease increases, greater public health efforts must be instituted to improve early detection efforts. Telemedicine efforts in this area range from efforts to improve screening to efforts to enhance early detection.

Building on behavior modification interventions for medication adherence, diet and exercise at Boston Medical Center, telehealth interventions are being used to enhance other reminders to obtain breast cancer screening. A telephone voice response system has been used to support mammography reminders among women 50–74 years of age. After the initial reminder letter, study participants receive an automated phone call with a recorded voice. The voice asks questions about barriers that the participant may have encountered

in obtaining a mammogram. Women respond by using the telephone's touch tone keypad. Women may then be asked subsequent questions or may be offered encouragement and support about the value of annual mammography (Hightower 2003). Web sites to encourage breast cancer early detection have also been developed (Zdravkovic and Striber Devaja 2002). These web sites are popular in the U.S. and worldwide; however, assessments of efficacy and quality assurance measures are difficult to implement on a broad scale. Efforts to guide consumer use of these web sites are critical to ensure that the information that reaches the patient is accurate.

Mammography remains the gold standard for early detection of breast cancer. Increasing access to telemammography services has long been a public health concern. In remote communities, mammography services may be provided by a mobile van that brings the mammograms to the mammographer, by a traveling mammographer who periodically reads films on site, or by a mammographer who receives the mammograms by courier. These factors contribute to the women experiencing longer wait times to receive the final mammography results. When results are available, a patient may not easily be found to receive her results. Women may not have a phone or may have moved, and therefore may not be reached to receive the results. If the patient is not receiving regular annual follow-up, abnormal test results may not be followed until after disease has advanced. This loss to follow-up can be devastating. Telemammography services may help to ameliorate these problems. Mammograms can be performed at a 'mobile' clinic and tele-transmitted for reads at a centralized mammography center, over a telecommunications network. The project, which was intended to enhance timeliness of service to the rural community, has far exceeded expectations and has had a major impact on women's health care in that community. By introducing an effective turnaround time of approximately 1 h, women willingly wait for their mammography results. This enhances cancer detection capacity by eradicating the need to track down patients who may have moved, who may not have a phone or who do not have an address but who now have an abnormal mammogram result. Since the conception of this service in Arizona, nearly 20,000 mammograms have been performed from distant sites (Lopez et al. 2008). Additional measures adding rapid tissue assessments are ongoing (Grogan et al. 2000; Lopez et al. 2006).

### 8.4.2 Cervical Cancer

Telecolposcopy involves the use of telemedicine technology to assess the cervix from a distance. Studies have been conducted to compare the efficacy and accuracy of telecolposcopy versus traditional colposcopy. In one study in a tertiary care clinic, participants underwent a clinical exam, an in-person exam, and a store-forward telecolposcopy exam (Lopez et al. 2004a; Lopez et al. 2004b). Different examiners then assessed the store-forward images. Analysis included comparison of the clinical and telemedicine diagnosis alone, and with biopsy results, interobserver and intraobserver correlations, and time to assess and satisfaction with the image. Results indicate that the sensitivity of telecolposcopy was significantly better than chance ($P < 0.001$). The positive predictive value of the colposcopic impression was highest for high-grade or invasive lesions and no cervical intraepithelial neoplasia. For the two physicians

reviewing telecolposcopic images, the positive predictive value of the telecolposcopic impression was 81% and 82%, respectively, while the positive predictive value of the in-person impression was 80%. Patients accepted the technology well and expressed no discomfort that images would be transmitted. The conclusion of the study was that telecolposcopy diagnosis agreed well with in-person colposcopy diagnosis (Lopez et al. 2004).

Subsequent work in a primary care clinic supports the feasibility for implementation and broader utilization (Lopez et al. 2004; Lopez et al. 2005). Work assessing and comparing synchronous real-time telecolposcopy with asynchronous computer evaluation are ongoing (Lopez et al. 2004). Although computer, store-forward assessments are less costly and result in better images (Harper et al. 2000; Ferris et al. 2003), visualization of the area in question and real-time clinical directions may be more effective with real-time telecolposcopy (Ferris et al. 2002, Ferris et al. 2004).

### 8.4.3 Skin Cancer

Teledermatology typically involves photos or videos of in-person dermatological consults that are assessed using store-forward teleconsultations. A study to compare the diagnostic accuracy of in-person versus store-forward teledermatologic consultations was undertaken (Krupinski et al. 1999). Three hundred and eight consecutive patients presenting to the University of Arizona's Dermatology Clinic participated in the study. Each patient was examined by one of three study dermatologists, each of whom examined approximately one third of the total number of patients. Digital photos were then obtained of the skin lesions from each of the patients. Each dermatologist rendered a teledermatology diagnosis, rated her or his level of confidence in the diagnosis, and rated her or his satisfaction with the image resolution and color. The viewing time for determining each diagnosis was also recorded.

Eighty percent agreement existed between in-person and digital diagnoses. Intradermatologist agreement averaged 84%, with slightly lower agreement when restricted to exact matches as opposed to matches with minor differences, which were not associated with different treatment. Confidence in diagnosis decisions was rated as definite or very definite across 70% of cases. Agreement between diagnosis and biopsy results averaged approximately 75%. Image resolution was rated as either good or excellent 83% of the time, and color quality was rated the same as in-person assessments in 93% of cases.

Oliveira and colleagues (Oliveira et al. 2002) have described the development of a web site to enable nonmedical health professionals to screen skin potentially malignant skin lesions. The nurse assistant photographed the lesions of 92 patients who presented some kind of dermatological condition. The images were then sent for evaluation by the dermatologist followed by in person examination by the same physician. The diagnoses, which resulted from the examination in person and, in some cases, the biopsy results, were compared with the diagnostic impressions of the nurse assistant and with the diagnostic hypothesis of the dermatologist at a distance. The lesions were classified as either malignant or nonmalignant. Kappa statistics showed a high association between the suspected malignancy and nonmalignancy of the lesions between the dermatologist ($p = 6.01 \times 10^{-9}$) and the nurse

assistant and between the diagnosis at distance and in person ($p < 1.0 \times 10^{-14}$). The web site allowed a nurse assistant to screen for potentially malignant skin lesions and, thus, proved to be appropriate for a large-scale test.

A study by Phillips and colleagues (Phillips et al. 1998) was conducted to determine the reliability of videoconferencing technology in evaluating skin tumors, the impact of the technology on the clinicians' degree of suspicion that a skin tumor is malignant, and the recommendation to do a biopsy. Four skin cancer screenings were conducted at rural health care facilities. A dermatologist saw the patients in person at the local facility, and the same patient was seen by a dermatologist using a high speed digital (T-1) connection to Greenville, North Carolina. The two physicians were in absolute agreement for 59% of the 107 skin tumors evaluated. There were five lesions identified by the on-site dermatologist as a probable or definite malignancy. The degree of concern about a lesion being malignant and the decision whether to do a biopsy were not significantly different, as shown by kappa analysis. The concern about the malignancy of a particular skin lesion and the recommendation whether to do a biopsy were not significantly affected by telemedicine technology.

The use of videoconferencing equipment for medical applications is undergoing evaluation in several centers in New Zealand. Patients with skin diseases who live in rural areas in New Zealand have no local access to specialist advice. The principal advantage of dermatology consultations using videoconferencing equipment is decreased travel time for the patient, although the costs of travel, time off work and domestic help are also reduced. In a case report from this New Zealand teledermatology clinic, rapid assessment of a skin lesion and image storage allowed immediate referral to the appropriate surgical service so that the patient received rapid care for what was determined to be melanoma (Oakley et al. 1996).

### 8.4.4 Colorectal Cancer

Colonoscopy is the examination of choice to assess premalignant and malignant lesions in the colon. Virtual colonoscopy is a safe, minimally invasive three-dimensional imaging method that may be an effective alternative especially in places where a colonoscopist may not be available. Pickhardt and colleagues (Pickhardt et al. 2003) evaluated the performance characteristics of computed tomographic (CT) virtual colonoscopy for the detection of colorectal neoplasia in an average-risk screening population. A total of 1,233 asymptomatic adults (mean age, 57.8 years) underwent same-day virtual and optical colonoscopy. Radiologists used the three-dimensional endoluminal display for the initial detection of polyps on CT virtual colonoscopy. For the initial examination of each colonic segment, the colonoscopists were unaware of the findings on virtual colonoscopy, which were revealed to them before any subsequent reexamination. The sensitivity and specificity of virtual colonoscopy and the sensitivity of optical colonoscopy were calculated with the use of the findings of the final, unblinded optical colonoscopy as the reference standard. The sensitivity of virtual colonoscopy for adenomatous polyps was 93.8% for polyps at least 10 mm in diameter, 93.9% for polyps at least 8 mm in diameter, and 88.7% for polyps at least 6 mm in diameter. The sensitivity of optical colonoscopy for adenomatous polyps

was 87.5% , 91.5%, and 92.3% for the three sizes of polyps, respectively. The specificity of virtual colonoscopy for adenomatous polyps was 96.0% for polyps at least 10 mm in diameter, 92.2% for polyps at least 8 mm in diameter, and 79.6% for polyps at least 6 mm in diameter. Two polyps were malignant; both were detected on virtual colonoscopy, and one of them was missed on optical colonoscopy before the results on virtual colonoscopy were revealed. CT virtual colonoscopy with the use of a three-dimensional approach is an accurate screening method for the detection of colorectal neoplasia in asymptomatic average-risk adults and compares favorably with optical colonoscopy in terms of the detection of clinically relevant lesions (Pickhardt et al. 2003). Testing and evaluation of acceptability of this intervention is ongoing.

Baca and colleagues (Baca et al. 2007) designed a study to determine the contributions of virtual colonoscopy to laparoscopic colorectal surgery. Virtual colonoscopy was performed in 40 consecutive patients who had undergone laparoscopic resection for colorectal neoplasm. Preoperative findings of optical colonoscopy and virtual colonoscopy, operative data, tumor localizations and histopathologic findings were assessed. Accuracy rates for virtual colonoscopy and optical colonoscopy were 97.5% and 55%, respectively ($P < 0.05$). Polypectomy site was localized with virtual colonoscopy in five patients. There were nine partially obstructing tumors that did not allow optical endoscope passage. Four of six synchronous tumors (one tumor and three polyps) couldn't be shown with optical colonoscopy because of distal obstructing tumor. Histopathologic investigations revealed adenocarcinoma (n = 34), adenoma demonstrating low-grade dysplasia (n = 3) and high-grade dysplasia (n = 2) and neuroendocrine carcinoma (n = 1). Mean tumor size was four centimeters. Mean proximal and distal surgical margins were 15 cm and 7.3 cm, respectively. Overall patient preference was 87.5% for virtual colonoscopy. Correct localization of colorectal neoplasm or polypectomy site is mandatory before laparoscopic colorectal surgery (Baca et al. 2007).

### 8.4.5 Prostate Cancer

It has been shown that diagnostic teleconsultation and quantitative image analyses via the Internet are not only feasible, but practical, and allow a close collaboration between researchers widely separated by geographical distance and analytical resources. In this study, 1,168 histological images of normal prostate, high grade prostatic intraepithelial neoplasia (PIN), and prostate cancer were recorded, archived in an image format developed at the Optical Sciences Center (OSC), University of Arizona, and transmitted to Ancona, Italy, as JPEG (joint photographic experts group) files (Montironi et al. 2002). Images were downloaded for review using the Internet application FTP (file transfer protocol). The images were then sent from Ancona, Italy to other laboratories for additional histopathological review and quantitative analyses. The three applications of the telecommunication system were remote histopathological assessment, remote data acquisition, and selection of material. There were only negligible transmission errors, and no problem in efficient communication, although real time communication was an exception, because of the time zone differences. As far as the remote histopathological assessment of the prostate was concerned, agreement between

the pathologist's electronic diagnosis and the diagnostic label applied to the images by the recording scientist was present in 96.6% of instances. When these images were forwarded to two pathologists, the level of concordance with the reviewing pathologist who originally downloaded the files was as high as 97.2 and 98.0%. Initial results of these studies made by researchers belonging to this group but located in others laboratories showed the feasibility of making quantitative analysis on the same images (Montironi et al. 2002).

### 8.4.6 Telepathology

Pathology diagnosis remains the hallmark of cancer diagnosis. As we develop a greater understanding of the preneoplastic process and its histopathologic characteristics along with the molecular biology markers present before the frank diagnosis of cancer, a greater need for centralized pathology services will emerge. Telepathology may likely serve as the means by which this need can be met (Weinstein et al. 1997). Telepathology was initiated as a means to increase access to frozen section results (Liang et al. 2008), second-opinion telepathology consultations, and quality control measures among remote or nonurban communities (Weinstein et al. 1987).

As the field develops, a greater need for specialized pathology reviews for oncologic diagnosis has emerged. To allow for these services, technology facilitating the translation of the glass slide to the tissue slide was critical. The digital slide can be manipulated and viewed at a higher power; all while preserving the original digital transmission. In addition, with the development of rapid tissue processing technology, pathology diagnoses may be rendered within a 24-h period (Weinstein et al. 2004). Ultrarapid virtual slide processing and telepathology are being paired with cancer diagnostic measure to provide earlier diagnosis, decrease anxiety associated with the wait time and facilitate earlier entry into the cancer treatment cycle (Grogan et al. 2000; Lopez et al. 2006).

## 8.5 Educating the Community Health Worker

*Promotoras*, or lay community health outreach workers, deliver breast cancer information to Hispanic women along the U.S.-Mexico border; however, uniform up-to-date training remains a challenge. A program was developed to deliver and assess the effectiveness of a sustainable breast cancer tele-education curriculum for *promotoras* in Southern Arizona (Lopez et al. 2007). The program curriculum was transmitted to four communities along the border. Topics included risk assessment, treatment, nutrition, community resources, clinical trials, and survivorship. The videoconferencing equipment utilized included a Tandberg Healthcare Unit III, a Tandberg 880, and a Polycom set-top unit. Sites were bridged together by engineers using an Accord Multi-Point Control Unit. Program assessment included satisfaction surveys, pre and post knowledge evaluation, 1-month interview and 4-month focus group. Of the 20 *promotora* participants, 14 identified themselves as

Latina, three identified themselves as White and two identified themselves as African American. All participants had at least 1 year of experience as a community health outreach worker. Satisfaction surveys indicate an overall high level of satisfaction for all sessions. Findings of the pre/post evaluation revealed improved understanding and knowledge of breast cancer definitions. One month follow-up data indicated that all participants had used the materials provided and that *promotoras* had developed an increased confidence in educating women about breast cancer. Four-month follow-up focus group revealed that the participants experienced increased access to health information and expressed that they felt that program materials had been extremely useful in their work. They perceived that they were better able to serve their community. Outreach tele-education to *promotoras* improved their skills and confidence as health educators with reported improved benefits to the community that they serve (Lopez et al. 2007).

## 8.6 Future Directions

Cancer prevention is a rapidly evolving field; however, a major limitation in its efficacy is the limited access that persons have to cancer preventive care. Housed largely in academic centers, cancer preventive services are unreachable to many. Telemedicine technology has demonstrated cost and clinical efficacy in many aspect of care including teleoncology services. Looking specifically at cancer prevention applications for telemedicine, efficacy is demonstrated for primary and secondary preventive efforts for all the screenable cancers. Increased development of cancer preventive services via telemedicine can serve to bring state of the art cancer preventive screening technologies and interventions to persons in remote or underserved communities, thus enhancing the health care of the population.

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# The Drug Development Process

# 9

**N.E. McKenzie**

The chemopreventive agent development process is a science in its infancy. Developers of chemopreventive agents face the same regulatory hurdles as do therapeutic drug developers. As in all drug development, chemoprevention scientists must demonstrate both safety and efficacy for an agent to be approved for marketing to the public. Historically, this has meant that developers must demonstrate reduced cancer incidence or mortality in order to show effectiveness. Needless to say, this would be a lengthy process given the 20–30-year trajectory of carcinogenesis. This requirement has been modified in recent years. The notion of targeted prevention of cancer is now based on the discovery of surrogate endpoint biomarkers, signal transduction pathways, and the ability to promote or inhibit specific molecules in those pathways with new molecular entities (NME) or drugs (O'Shaughnessy et al. 2002).

In the United States (US) and internationally, the development and manufacture of drug products is regulated by government entities in order to protect general populations as well as research participants. In the US, the Food and Drug Administration (FDA) has this regulatory responsibility, while in Europe and Japan, the responsibility falls to individual governments using standards established and maintained by the International Conference of Harmonization (ICH). The regulatory agencies and the developers of drugs must balance the benefit of new drugs to the population as a whole against the risk to individuals participating in clinical trials and eventually to the general public. In the development of chemopreventive agents, the risk must be very low and the benefit very high, as, in order to be effective, large at-risk populations would need to use the drug, possibly for life, in order for a drug to be effective in preventing cancer (Anonymous 1999).

The process of developing chemopreventive agents consists of several systematic steps. First, NMEs are chosen based on basic science findings. Promising agents then undergo preclinical testing in animal models. Before human testing can begin, the science must be reviewed by the FDA or other regulatory agency. After the completion of clinical trials and prior to marketing, findings must be evaluated and communicated to the scientific

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community. This chapter outlines the process of developing chemopreventive agents and the standards that guide such development.

## 9.1 Selecting New Molecular Entities for Development as Chemopreventive Agents

New molecular entities are the focus of scientific study in cancer therapeutics as well as cancer prevention programs across the world. However, the selection of new molecular entities (NMEs) for development is not a random or serendipitous process. Rather, specific important criteria apply to the selection of NMEs for clinical development as potential chemopreventive agents. The major criteria include evidence of activity in preventing cancer at the target site, low toxicity to allow for potential use in large populations, the identification of the biomarkers associated with the effectiveness of the NME, and the availability of a pertinent population for clinical testing. The focus of these criteria is the real feasibility of giving a drug prophylactically to large populations at risk for a particular type of cancer (Kelloff et al. 1996).

There are significant precedents for the notion of developing drugs that lessen the risk for a disease or the recurrence of disease and that can be given on a large scale. An example of such a precedent is the development and FDA approved marketing of lipid lowering drugs that aim to reduce the risk of cardiovascular disease. The process included research on cholesterol lowering and modulation of other markers. Lowering of cholesterol emerged as a definitive risk reduction marker and development of cholesterol lowering drugs followed. In the case of cancer chemopreventive agents, early associated biomarkers discovered by basic scientist must also form the basis for development. Examples of such markers are proliferation antigens [e.g., proliferating cell nuclear antigen (PCNA)], inhibition of growth factors [e.g., epidermal growth factor receptor (EGFR)] and apoptosis.

Discovery of related biomarkers is followed by research to demonstrate activity against proliferation of pre-cancer or intraepithelial neoplasias (IEN). Demonstration of activity is performed in vitro in cell lines and later in vivo in animal models. Satisfactory activity in both models can then be evaluated as justification for proceeding to clinical trials. In addition to peer review by other scientists, the FDA performs the function of approving clinical trials in humans using NMEs. The criteria used by the FDA to evaluate proposed drugs for development and marketing are safety and effectiveness viewed in a risk-benefit balancing framework.

An important requirement for chemoprevention agents is that while they must be highly effective, they must also be safe enough to be given to large populations at risk for a particular cancer. This is a marked departure from the usual thinking about therapeutic cancer drugs where the toxicity, unless major, is an expected fact that is mitigated by the effectiveness of the drug in treating the cancer. Therapeutic clinical trials, therefore, tend to seek the highest effective dose for which the toxicity can be justified by its effectiveness, while chemoprevention studies seek to find the highest safe dose that is also effective.

An important consideration for selecting a NME for development as a chemopreventive agent is the availability of the agent in quantities needed for all phases of testing. A supply can be assured by either synthesizing the drug, provided no patent rights are contravened, or acquired in bulk from the manufacturer or wholesaler of known compounds. Synthesis can be a computer assisted process in which a compound is simulated using enzymes

that attach to the disease related target site on the cell membrane. Synthesis of a drug for research also requires attention to documentation of good laboratory practice (GLP). While formal GLP is not required for synthesis of drugs intended for laboratory use only, it becomes a consideration when applying for FDA approval for testing in humans.

Acquisition can be a more efficient way of getting a supply of a new drug substance for testing. Many known compounds are available from chemical suppliers and even proprietary drugs may be available in sample quantities or by agreement from the manufacturers. Academic institutions can be highly effective and less costly than commercial laboratories in basic and preclinical science portions of drug development. Such arrangements are usually managed by Materials Transfer Agreements (MTA). The FDA may require that a compound that is to be used in clinical trials be manufactured under current Good Manufacturing Practice (GMP). It may therefore be necessary for clinical researchers to limit drug development to the pre-clinical stage unless a GMP supply of the study compound can be found.

Some promising chemopreventive compounds may have been tested in countries where laws are less restrictive. Drugs thus studied may be of interest to drug development researchers in FDA or ICH regulated countries and may enter their list of potential drugs as a result of preclinical activity or safety and effectiveness in humans. However, any human testing must be repeated under US regulatory requirements. Under new guidance for botanicals, clinical data obtained in non-US safety trials can be used to support later and larger trials provided the botanical used is the same. However, the developer must be able to show bioequivalence. Likewise, agents may have been tested for other purposes and based on other activity and have subsequently yielded serendipitous findings. Repeating studies designed to explore those findings can lead to discovery of safety and effectiveness for those new indications. In all cases, scientifically sound animal testing must precede testing in humans in order to get a preliminary idea of what may be expected in regard to safety and effectiveness in humans and to get information to guide dose setting in clinical trials. In summary, agents selected for clinical trials are those which have shown the most activity in preclinical testing and at the same time have shown the least toxicity at doses that can be tolerated by large elements of the population.

## 9.2 Regulatory Requirements and the US Food and Drug Administration

Details of the legislative basis of the drug development regulatory authority of the US Food and Drug Administration (FDA) and the requirements of the regulatory process are the basis for an understanding of the Investigational New Drug application (IND) and the accepted phases of clinical trials research. Good Clinical Practice considerations are essential for drug trials in human and the requirements for the protection of human subjects in clinical trials. Specifics of the regulatory requirements outlined here can be found in the current Code of Federal Regulations, Title 21, Parts 50, 54, 56, and 312.

The FDA, an agency within the Department of Health and Human Services (DHHS), is authorized to regulate drug development by a series of federal laws. These laws, enacted over the past 150 years, are authorized under the Interstate Commerce Clause of the US Constitution. The first law enacted, the Drug Importation Act of 1848, was written in

response to the perception that excessive mortality during the Mexican War was caused by harmful drugs entering the country and that such substances had to be regulated or prohibited. Virtually every subsequent law was passed in response to an actual or perceived problem. The Biologics Control Act was passed in reaction to a diphtheria vaccine developed from horses with tetanus that caused the death of many children, especially in St. Louis. This Act was designed to ensure purity and safety of serums and vaccines and included annual licensing requirements and inspections of manufacturing facilities (FDA 1999).

In 1906, the Pure Food and Drugs Act was passed as the foundation of modern food and drug law. It was, in large part, the creation of Harvey Washington Wiley, the chief chemist of the Bureau of Chemistry. The Act prohibited interstate commerce of mislabeled and adulterated drugs and food. The basic authorizing act of the FDA came in 1938 in the Federal Food, Drug and Cosmetic Act. Again, it was a reaction to an elixir of sulfonamide marketed by S.C. Massengill which used a solvent similar to antifreeze and caused the death of 107 people. It required new drugs, devices and cosmetics to be shown to be safe before they were brought to market. This Act required the filing of a New Drug Application (NDA) and adequate directions for safe use. Various refinements followed.

The next reactive legislation came about due to the thalidomide tragedy. Senator Estes Kefauver was holding hearings pertaining to the manufacturing of antibiotics and other development practices. An NDA was submitted for Kevadon, the brand of thalidomide that the William Merrell Company hoped to market in the US. Despite ongoing pressure from the firm, the FDA's medical officer, Dr. Frances Kelsey, refused to allow the NDA to become effective because of insufficient safety data. By 1962, thalidomide's horrifying effects on newborns became known. Even though Kevadon was never approved for marketing, Merrell had distributed over two million tablets for investigational use, use which the contemporary law and regulations left mostly unchecked. Once thalidomide's deleterious effects became known, namely major birth defects, the agency moved quickly to recover the supply from physicians, pharmacists, and patients. Henceforth, drug developers are required to provide sufficient preclinical data that a drug is safe to give to humans before commencing clinical trials. For her efforts, Dr. Kelsey received the President's Distinguished Federal Civilian Service Award in 1962, the highest civilian honor available to government employees.

Current regulations are contained in a series of laws including the FDA Modernization Act of 1997, renewed in 2002. Today, the FDA is responsible for the review of new products, keeping watch over the safety of the drug supply, developing standards and regulations, and correcting or preventing problems through regulatory compliance programs. Drug regulatory efforts are handled by the Center for Drug Evaluation and Research (CDER). The main areas of focus are safety, effectiveness and availability (FDA 1999).

## 9.3 The Investigational New Drug Application

An Investigational New Drug (IND) Application is required by anyone planning to test a new molecular entity in humans. An IND actually allows a developer to proceed by permitting the shipment of drug for the purpose of conducting clinical trials. The provisions of the Code

of Federal Regulations (CFR) that pertain to INDs are found in Title 21 Sect. 312 [21 CFR Part 312 (1999)]. An IND is not needed to study a legally marketed drug, unless the studies will deal with new indications, new delivery methods or new labeling claims. This section is a global review of this process and is not intended to provide comprehensive information for drug developers to file an IND. For more specific details, please consult the CFR.

The FDA's primary IND review objective is to protect human subjects involved in initial clinical trials. In later phases of investigation, the FDA also reviews IND submissions for scientific rigor to permit assurance of effectiveness. In addition to information about the applicant's identity and qualifications, the IND must contain the following information:

- A general investigational plan
- An investigator's brochure
- Protocol for planned studies (Nestle et al. 1998)
- Chemistry, manufacturing and control information
- Pharmacology and toxicology information
- Summary of previous human experience

In a general investigational plan a developer provides background information on the drug to be tested and the rationale for testing it in the manner and for the duration proposed. The developer also indicates the number of research participants to be enrolled over the course of the development of the drug. Most importantly, the developer summarizes the likelihood and severity of risks anticipated in humans based on the results of testing in animals.

The investigators brochure (IB) is a set of documentation that provides detailed information to investigators who will be performing the clinical trials. The IB includes information on the drug substance and how it is formulated. It summarizes pharmacological and toxicological effects and pharmacokinetics of the drug as tested in animals and discloses any such findings available in humans and concludes with a discussion of anticipated risks, special precautions and monitoring needed in the clinical trial.

Protocols must contain the objectives of each proposed study with specific attention to the indications studied and desired endpoints. Protocols must be specific in detailing the number and characteristics of study participants to be recruited and those to be excluded for safety reasons. The design of the study must be described in detail and must be appropriate to the type of trial envisioned. In particular, details pertaining to randomization, blinding and control are specified in the protocol. Finally, the protocol must describe what the actual data will look like and how they will be analyzed and reported. The data collected must include specific measurements pertaining to the safety of study participants, so that adverse events related to the administration of the drug can be reported and evaluated.

Chemistry, manufacturing and control (CMC) information includes relatively complex information about the composition, manufacture and quality control of the drug substance (raw drug) and drug product (drug as formulated for use in humans). Identity, quality, purity and strength of the drug must be supplied to the FDA as well as the methods for validating each parameter. The FDA must be assured by the manufacturer that these parameters will remain stable over time and can be maintained from one batch to the next. As the scope of investigations increases, the FDA requires that new information pertaining to CMC be submitted in detail.

Pharmacology and toxicology information is very important in the IND as this is the basis for concluding that the drug can be reasonably assumed to be safe to study in humans. Pharmacology information includes the effects and mechanism (Nestle et al. 1998) of action of the drug in animals. To the extent known, the absorption, distribution, metabolism and excretion of the drug are included in this section. Toxicology information consists of an integrated summary of toxicological testing in animals. Specifically, results of acute, sub-acute and chronic toxicity testing in animals are reported as are reproductive or developmental effects of the drug. Full tabulations of data, not summaries, must be provided to the FDA.

If previous human studies have been performed with the drug, for example, by another route or for different indications, such data are to be summarized in the IND as should any human trials experience from other countries. Any published papers can be included in the submission. Certainly, if the drug to be studied is marketed or has previously been marketed in another country, experience from that population should also be included in the application. Finally, for drugs that are believed to have abuse potential, are radioactive or are to be studied in a pediatric population, submission of additional information illuminating those characteristics, as applicable, is required.

## 9.4 Phases of Clinical Research

The Phases of Investigation are a set of conventions the FDA requires developers to use when planning and reporting clinical trials. There are four phases of investigation, of which the first three are most commonly used. Phase I represents the first use of a given drug in humans. This kind of study is usually performed in a small sample, in the range of 20–80, and with normal volunteers, but it can also be done with patients. In this phase of study, the drug is tested in humans to establish a measure of safety and to provide enough information about metabolism and the pharmacokinetics of the drug to design a well-controlled, scientifically valid Phase II study. Phase I studies are typically closely monitored, especially if no previous experience in humans exists.

Phase II studies include studies to evaluate effectiveness for a particular indication in patients with the disease as well as continuing the evaluation of safety. Phase IIa studies are usually small and fairly short in duration, typically three months, and are designed to evaluate dose-response on both pharmacological and intermediate endpoints. Phase IIb studies are randomized, placebo-controlled, double-blinded trials in a larger group of patients, perhaps 75–100. In this phase, participants usually receive the same dose, possibly with dose-reduction provided for in the event of adverse study related effects. The investigational treatment may also be compared to standard treatment in this phase. Endpoints of Phase II trials in chemoprevention studies would include the biomarkers and pathways found to be affected in pre-clinical and animal studies.

Phase III studies are larger trials of more than 100 participants. This phase of study is specifically aimed at determining whether the new drug is more effective and/or safer than the standard treatment. Frequently, Phase III trials are conducted in multiple settings and locations across the country in order to supply evidence for effectiveness in a more

heterogeneous population. Generally, participants are randomized to an investigational group, which receives the test agent, or a control group, which receives the standard treatment. Phase III trials data are the centerpiece of the New Drug Application (NDA) submitted to the FDA to obtain permission to market the new drug.

Phase IV studies are undertaken after approval of the NDA and marketing of the drug. These studies, which are not as common as Phase I through III trials, can be requested by the FDA as non-essential to the approval of the drug, but useful in providing additional information that could, for example, change the prescribing information or labeling of a drug. If requested, manufacturers commit to do such studies at the time of the approval of the NDA. The design of Phase IV studies depends on the research question. They generally resemble Phase III studies but participants come from a more defined population in order to support specific use of the drug. Phase IV studies can also be undertaken voluntarily by manufacturers to extend the marketing potential of the drug.

## 9.5 Good Clinical Practice

The term Good Clinical Practice (GCP) refers to a standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity and confidentiality of trial subjects are protected (ICH 1994). Developed by the International Conference on Harmonisation (ICH), GCP guidelines follow a set of fundamental principles for the ethical conduct clinical trials involving human subjects. These principles were formulated in the World Medical Association's Declaration of Helsinki in 1964 and revised in 1989 (WMA 1989). The principles cover the manner of conducting clinical trials as follows:

- Clinical trials should be conducted according to ethical principles based on the Declaration of Helsinki.
- Risks should be weighed against potential benefits prior to starting a trial.
- The rights, safety and well-being of study participants are primary considerations.
- All available clinical and non-clinical information should be presented for evaluation of a proposed trial.
- Trials should be scientifically sound and clearly described in a detailed protocol.
- Protocol should be approved by an ethics board and should be followed exactly as approved.
- Any medical care given to study participants should be performed by or under the direct authority of a qualified physician.
- Individuals involved in conduction clinical trials should be qualified by education, training and experience to perform their study tasks.
- Freely given informed consent should be obtained from every study participant.
- All trial information should be documented and stored in a way that assures accurate reporting, interpretation and verification.

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- Investigational products should be manufactured, handled and stored according to approved standards.
- Quality assurance systems must be in place to assure quality of data.

Adherence to the principles of GCP is the responsibility of the sponsor, the principal investigator, and all study staff. Compliance is monitored by the FDA's Bioresearch monitoring department (BIMO). In the US, penalties for non-compliance can range from voluntary correction of deficiencies to fines and imprisonment in cases of deliberate fraud.

## 9.6 The New Drug Application

The New Drug Application (NDA) is submitted when all phases of investigation have been concluded. The NDA requires all the documentation necessary to present a complete picture of how the drug is made, formulated and checked for quality, how the drug performed in clinical testing, how the drug was tested in animals, and what happens to the drug in the body. The NDA also must include the proposed labeling so the FDA can ascertain that the claims made on the label do not exceed what can be reasonably supported by the clinical trials. Actual data and reports are part of the NDA so conclusions can be validated. The primary purpose of review is to evaluate safety and effectiveness in the context of the data presented and the claims made in the proposed marketing materials. The experiences gained in clinical trials provide the basis for telling health care providers how they should prescribe the drug. All this information is summarized on what becomes the package insert. If the documentation submitted to the FDA for review as an NDA is deemed insufficient, the application will be declined (FDA 1999). The company can then resubmit when the deficiencies have been addressed. Pharmaceutical companies pay a fee for the review of their NDA. Instituting this fee has shortened review times considerably and consequently has lessened the time it takes for new drugs to reach the consumer.

Clinical trials serve to promote safe and effective drugs and to eliminate those that turn out to be ineffective or too risky for the benefit they provide. Of the drugs tested for safety in Phase I, about 70% are successful. Of those, only about 33% successfully clear the effectiveness requirements of Phase 2. Of the drugs that successfully make it through Phase II, only about 25% make it through Phase III. In all, only about 20% of all INDs submitted are approved for marketing.

## 9.7 Conclusion

In this chapter we have reviewed the steps that are taken to develop a chemopreventive agent, from bench to market. While chemopreventive agents differ from therapeutic agents in their scope and intent, their development requires the same stringent adherence to laws, regulations and principles.

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# Developing Topical Prodrugs for Skin Cancer Prevention

# 10

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The skin plays multiple roles in protection from environmental insults yet skin damage, particularly that derived from sunlight, constitutes a major public health problem. End stage skin damage in the form of non-melanoma skin cancers (NMSC) are the most frequent malignancies in the United States with more than 1,000,000 cases diagnosed annually (Karagas et al. 1999). Melanoma skin cancer is the most rapidly increasing cancer. Actinic keratosis (AK), skin lesions that can progress to NMSC are far more prevalent than skin cancers. The occurrence of DNA damage and cellular responses to DNA damage are major determinants of skin damage including skin cancer (Ames 2001; Ullrich 2002). A compelling body of evidence now indicates that there are multiple targets for reducing skin damage and that several key micronutrients are candidates for skin damage prevention. However, a major challenge for the development of prevention strategies for skin damage is the difficulty of delivering micronutrients to skin. Delivery to skin via the blood circulation of nutrients taken orally is inherently inefficient since this delivery is distal to other organs, particularly the liver, which removes many agents by first pass metabolism. In addition the major cell targets for prevention of skin cancer are located in the epidermis, which is non-vascular.

Described here are strategies to limit skin damage and thus skin cancer by targeting multiple mechanisms that include preventing DNA damage, enhancing DNA repair, preventing immune suppression, and preventing migration of transformed cells from epidermis to dermis. Further, an approach for delivery of key protective agents to skin cells using prodrugs specifically tailored for topical delivery is described. Finally, this approach is illustrated using niacin as a model micronutrient demonstrating that topical delivery of this polar compound to skin cells via prodrugs is feasible and that targeted delivery provides prevention benefit for skin.

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## 10.1 Strategies for Intervention

Genotoxic stress is known to be a major factor in skin damage. While the mechanisms that cause skin damage are complex and incompletely understood, genotoxic stress in the form of DNA damage is a major factor. Figure 1 shows the primary sources of genotoxic stress in both the dermis and epidermis of skin and the consequences of this stress. Three interrelated sources of genotoxic stress in skin are reactive oxygen species (ROS), reactive carbonyl species (RCS) and sunlight. Sunlight is the major source of skin damage as it leads to DNA damage directly via formation of pyrimidine dimers and other photoproducts (Ullrich 2002) and indirectly via generation of ROS and RCS by photooxidation and photosensitization reactions (Wondrak et al. 2002a,c; Roberts et al. 2003). Indeed, sunlight has been documented as a complete carcinogen. While the UVB region of sunlight, the region responsible for most of the direct DNA damage by sunlight, is the most effective at initiation of squamous cell carcinoma (SCC), recent studies have shown that solar simulated light containing more predominantly UVA rays that induce ROS also cause SCC formation (Pentland et al. 1999; Agar et al. 2004). The involvement of ROS in the promotion and progression phases of skin cancer is well established (Perchellet and Perchellet 1989). ROS include superoxide, hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite, and hypochlorite. All cells are exposed to ROS during the normal course

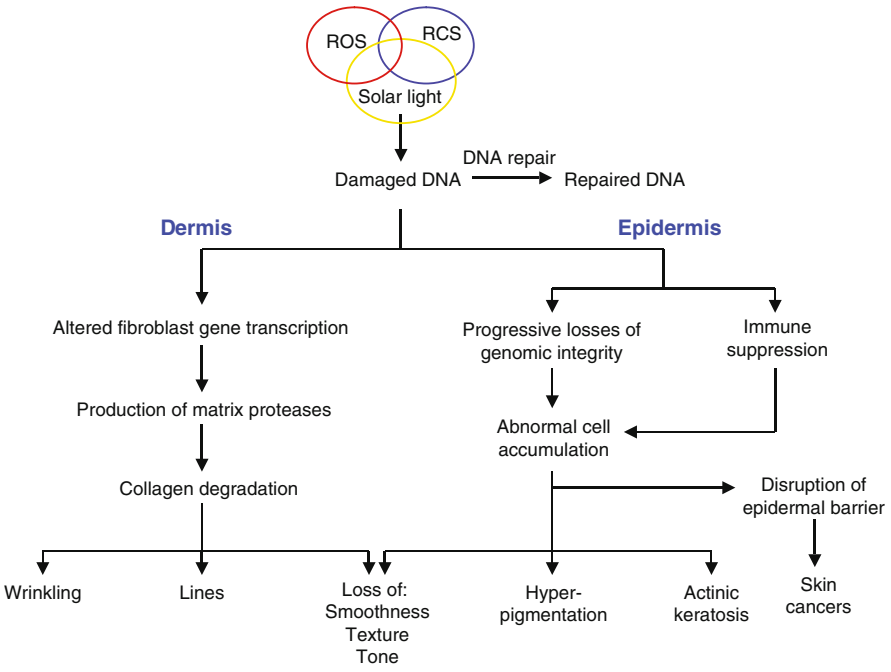


**Fig. 1** Central role of DNA damage in skin damage

of energy metabolism and and/or immune surveillance in addition to sunlight exposure. While ROS are involved in normal cell signaling pathways, increased ROS formation during oxidative stress disrupts signaling pathways causing negative consequences for normal cell function. In addition to DNA, proteins also are targets for damage by ROS in skin. Carbonyl stress, mediated by RCS from metabolic sources, lipid peroxidation, and glycoxidation targets skin cell DNA and extracellular matrix proteins with accumulation of protein advanced glycation end products (AGEs) during chronological and actinic aging of skin (Wondrak et al. 2002a,b,c; Roberts et al. 2003). Recently AGEs have been identified as potent UVA sensitizers of photooxidative stress in human skin, establishing a vicious cycle of RCS and ROS formation in sunlight induced genotoxic stress.

Figure 1 also overviews two major consequences of genotoxic stress in skin. First, chronic DNA damage results in progressive losses of genomic integrity that result in and are required for end stage skin damage in the form of skin cancer. These progressive losses of genomic integrity lead to altered growth properties of damaged keratinocytes such as unresponsiveness to terminal differentiation signals leading to epidermal hyperplasia and progressively to detectable skin lesions diagnosed as actinic keratosis (Jeffes and Tang 2000; Lober et al. 2000). Cell populations present in actinic keratosis lesions can progress to transformed cell populations that represent epidermal carcinoma in situ (Horowitz and Monger 1995; Guenthner et al. 1999). Subsequent cellular changes occur including induction of matrix proteases that facilitate disruption of the integrity of the epidermal barrier leading to invasion of the dermis, the point at which the damage process is diagnosed as SCC. A second major consequence of DNA damage in skin is the suppression of immune responses that would normally detect and remove damaged cells. While mechanisms of immune suppression extend well beyond DNA damage, the latter represents a major factor in immune suppression. The consequences of genotoxic stress include altered migration and antigen presentation by Langerhans cells, stimulation of cytokine release by keratinocytes that likely alters cytokine signaling required for normal immune surveillance including generation of T suppressor cells. Given the complexity of damage pathways and the down stream consequences, it seems likely that a combination strategy to prevent skin damage will be essential.

Figure 2 identifies opportunities for epidermal intervention by various agents where substantial evidence suggests the possibility of modulating the consequences of genotoxic stress. These opportunities include preventing DNA damage, enhancing DNA repair, preventing immune suppression both by preventing DNA damage and by mechanisms downstream from DNA damage, and strengthening the integrity of the epidermal barrier to prevent migration of transformed cells from the epidermis.

## 10.2 Strategy 1: Preventing DNA Damage

*Sunscreens*. The value of sunscreens in preventing DNA damage has clearly been demonstrated in animal models in which sunscreens applied prior to solar simulated light (SSL) exposure prevented p53 mutations and dramatically reduced skin cancer incidence

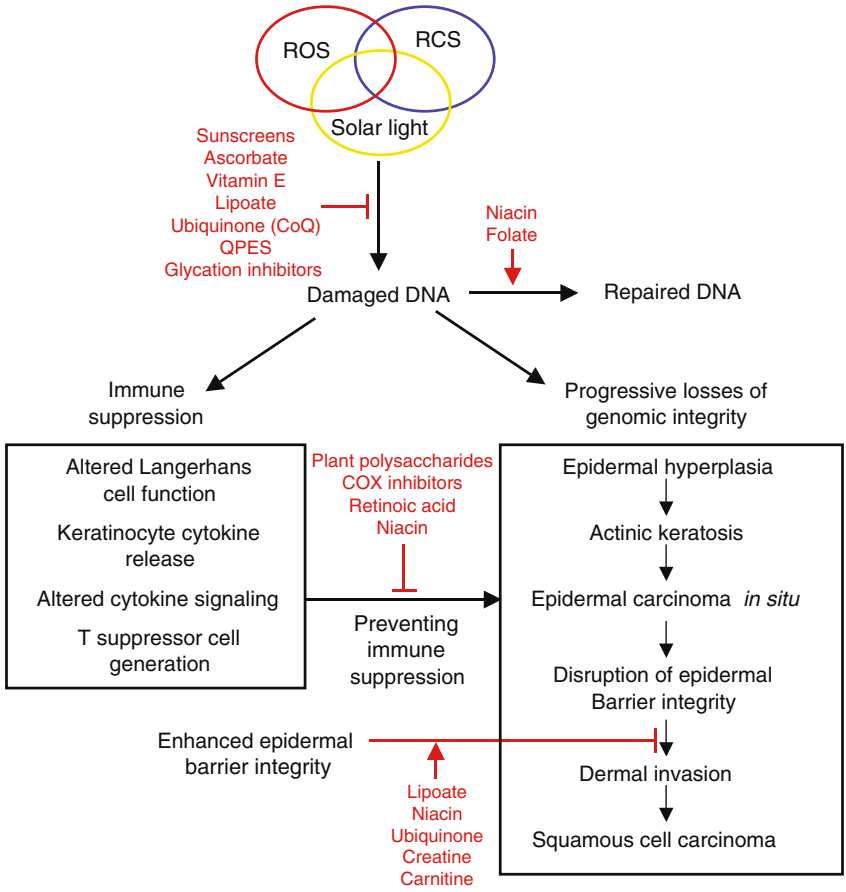


**Fig. 2** Opportunities for treatment and prevention of skin damage

(Ananthaswamy et al. 1999). Further, sunscreens prevent photoimmune suppression in mice (Reeve et al. 1998) and man (Fourtanier et al. 2000). Despite growing use of sunscreens, their inability to protect across a broad spectrum of solar radiation combined with poor public knowledge of appropriate selection and use have not led to decreases in skin cancer incidence. While the use of sunscreens needs to be an integral part of an overall strategy to reduce skin damage, their inability as a single agent to reduce skin damage illustrates the need for a combination of prevention agents.

*Ascorbate (Vitamin C)*. Vitamin C has been used widely in recent years as a skin protective agent. It is known to function as an antioxidant and likely serves multiple roles in collagen synthesis (Geesin et al. 1988). Further, this vitamin is important in recycling of reducing power in cells by exchange of electrons with Vitamin E (Beyer 1994). A study in mice has shown inhibition of phorbol ester induced skin tumor promotion using a lipophilic ester

of Vitamin C which, in its free form, is unstable and has poor penetration through the stratum corneum (Smart and Crawford 1991). Further studies of stable, deliverable forms of Vitamin C designed for optimal uptake by cells may prove beneficial in limiting DNA damage and improve skin cancer prevention. It is important to consider, however, that this compound can serve both as an antioxidant and a pro-oxidant.

*Tocopherol (Vitamin E)*. Topical application of tocopherol has been shown to decrease the incidence of ultraviolet-induced skin cancer in mice (Berton et al. 1998; Burke et al. 2000). Vitamin E provides protection against UV-induced skin photodamage through a combination of antioxidant and UV absorptive properties (McVean and Liebler 1997). Topical application of alpha-tocopherol on mouse skin inhibits the formation of cyclobutane pyrimidine photoproducts (Chen 1997). However, topically applied alpha-tocopherol is rapidly depleted by UVB radiation in a dose-dependent manner (Liebler and Burr 2000) as Vitamin E in skin can absorb UV light and generate the tocopheryl radical (Kagan et al. 1992). Hence, Vitamin E in skin may act in two conflicting manners, as a radical scavenger and possibly as a photosensitizer. Indeed, tocopherol has been shown to exacerbate UVA induced DNA damage in vitro (Nocentini et al. 2001). However, reductive antioxidants (e.g. ascorbate, thiols, ubiquinols) can reduce tocopherol radicals back to tocopherol (Kagan et al. 1990a,b). Unlike the soluble vitamins that are too hydrophilic for optimal delivery through the stratum corneum, Vitamin E is more lipophilic than is optimal for delivery into skin. Thus, when applied topically as the parent compound, residence time on the surface of skin is prolonged making the agent susceptible to UVB light absorption and possible conversion to a tocopheryl radical, which is a potential photosensitizer. On the other hand, when Vitamin E is stabilized by derivatization to a prodrug and effectively delivered into skin possessing an environment of reductive antioxidants (ascorbate, thiols, ubiquinols, etc) or formulated with antioxidants, tocopherol radical formation can be eliminated by three mechanisms: (1) decreased exposure to UVB on the surface of skin due to rate of delivery, (2) stability of Vitamin E due to derivatization to prodrug, and (3) rapid conversion back to tocopherol of any tocopheryl radicals formed due to the presence of reductive antioxidants in skin cells and/or in the delivery vehicle. While preclinical data demonstrate that tocopherol has photoprotective properties, clinical data do not yet convincingly show that dietary supplementation is of significant therapeutic value in protection from acute or chronic photodamage. Further, use of ester derivatives of Vitamin E to date have stabilized the molecule, but have increased the lipophilicity of the compound thereby decreasing its delivery to skin. This illustrates the general lack of understanding of delivery mechanisms for micronutrient benefits in skin. Thus, it seems likely that cutaneous bioavailability of dietary and existing preparations of topical tocopherol may be insufficient to combat photodamage in skin.

*Lipoate*. While a clear role for direct ROS scavenging by lipoate has not been firmly established, lipoate has a redox potential of −0.32 V, allowing it to reduce oxidized glutathione and ascorbate non-enzymatically in the skin antioxidative network (Guo and Packer 2000). In keratinocytes, lipoate is reduced to its active form, dihydrolipoate, which results in significant inhibition of the consumption of tocopherol and ubiquinone following UVA irradiation (Guo and Packer 2000). This protection presumably occurs via the role of lipoate along with ascorbate and tocopherol in the maintenance of redox balance. Lipoate topically applied to hairless mouse skin shows penetration and conversion

to dihydrolipoate demonstrating cellular delivery although the efficacy of delivery was not examined in detail (Podda et al. 1996). Lipoate per se is much too hydrophilic for effective topical delivery.

*Ubiquinone (Coenzyme Q10)*. Ubiquinone offers the potential to reduce DNA damage directly as an ROS scavenger and by supporting redox cycles that resist oxidative stress (Tomasetti et al. 1999). Ubiquinone and tocopherol are the major lipophilic antioxidants in skin (Shindo et al. 1994). The content of ubiquinone is nine times higher in the epidermis than in the dermis and a strong role in protection against skin damage is suggested by the observation that skin ubiquinone content decreases rapidly following solar irradiation (Shindo et al. 1994). A recent study has demonstrated that in vivo supplementation with ubiquinone enhances the recovery of human lymphocytes from oxidative DNA damage, supporting the hypothesis that this micronutrient can limit DNA damage in vivo (Tomasetti et al. 1999). In addition to its role as a direct ROS scavenger, ubiquinone has been postulated to function as an integral part of antioxidant defense pathways that also include tocopherol, ascorbate, glutathione and NADPH (Podda and Grundmann-Kollmann 2001). Beneficial effects of topical ubiquinone on prevention and reversal of skin photoaging also have been reported although the same study reported that the extremely lipophilic nature of the molecule strongly limited bioavailability following topical application (Hoppe et al. 1999), again illustrating the desirability of an effective strategy for topical delivery of this micronutrient. As with the case of tocopherol, ubiquinone per se also is much too lipophilic for effective topical delivery.

*Quenchers of Photoexcited States (QPES)*. We have coined the term QPES to refer to agents that physically quench or dissipate the energy transferred from sunlight to skin molecules, thereby inactivating photoexcited states that would ultimately interact with oxygen or other molecules to produce RCS and ROS, hydrogen peroxide and singlet oxygen in particular. UV and near visible chromophores in skin extracellular proteins (keratin, collagen, and elastin) are endogenous photosensitizers that mediate photodamage in human skin (Wondrak et al. 2002c, 2003). Small molecule quenchers of this novel class of sun damage targets are predicted to serve a chemopreventive role in suppressing photooxidative pathways of photocarcinogenesis and photoaging by direct physical quenching of photoexcited states that occurs without chemical depletion or need for metabolic regeneration of the active compound. This represents intervention at a very early step in the production of these UV induced deleterious species upstream of ROS formation. We have identified small polar compounds that accomplish these goals and envision that derivatives suitable for topical delivery will be required for optimal protective benefit to limit skin damage.

*Glycation Inhibitors*. Among the chromophores in skin that serve as photosensitizers of UV light are the non-enzymatically formed AGE chromophores generated from chemical reactions between reducing sugars and other RCS with protein amino groups followed by rearrangements and oxidation reactions. These structures form during intrinsic aging and accumulate at accelerated rates in photoaged skin. Interaction of AGEs with UV light readily generates ROS and more RCS forming a vicious cycle of skin damage (Wondrak et al. 2002a,b,c; Roberts, Wondrak et al. 2003). Identifying agents to inhibit the formation

of AGEs and optimize delivery of such agents could be very beneficial in limiting DNA damage that contributes to skin cancer.

## 10.3 Strategy 2: Enhancing DNA Repair

*Niacin*. A major target for niacin (nicotinic acid) with regard to enhancing DNA repair is poly(ADP-ribose) polymerase-1 (PARP-1) and downstream signaling pathways, whose activity is enhanced by niacin due to increased availability of NAD, the bioactive form of niacin and a substrate for PARP-1. The involvement of PARP-1 as a target for cancer prevention by niacin is based on studies that have demonstrated the involvement of PARP-1 in the maintenance of genomic integrity following genotoxic stress (Jacobson and Jacobson 1999; Rolli et al. 2000). PARP-1 functions in the synthesis of chromatin-associated polymers of ADP-ribose that function in cellular recovery from DNA damage and maintenance of genomic stability. The activation of PARP-1 by DNA strand breaks leads to complex signaling pathways that can enhance cell survival or result in cell death by apoptosis as shown in Fig. 3. In cases where the amount of damage is relatively small, PARP-1 activation enhances cellular recovery by interaction with other proteins such as p53 and the nuclear proteosome to stimulate both DNA repair and histone degradation such that the cell can fully recover from the genotoxic stress. When the damage is relatively higher, PARP-1 plays a key role in effecting cell death by apoptosis through its transcriptional activation role involving the NF-kB pathway and by preventing ATP depletion and DNA repair through PARP-1 cleavage (Jacobson and Jacobson 1999). Of direct relevance to

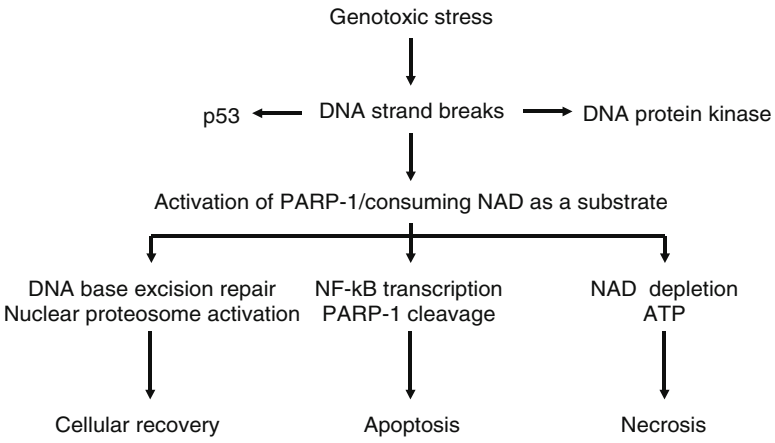


**Fig. 3** Interrelationship of niacin metabolism and DNA damage and repair pathways

skin, PARP-1 has been shown to be required for Fas, FasL mediated apoptosis critical to removal of badly damaged and potentially carcinogenic "sunburn" cells that arise following sunlight exposures that lead to erythema (Hill et al. 1999). Validation of niacin as a chemoprevention agent has been obtained in a mouse model where high dose oral niacin intake resulted in dose-dependent (1) increased skin NAD content, (2) decreased skin tumor incidence 70%, and (3) and reduced immune suppression 86% (Gensler et al. 1999).

*Folate*. A major prevention target for folate relates to its role in providing precursors for DNA repair synthesis. It may also promote genomic integrity through its role in the generation of methyl groups needed for control of gene expression. Its cancer protection potential has been demonstrated by large-scale epidemiological and nutritional studies indicating that decreased folate status increases the risk of developing stomach (Fang et al. 1997), colorectal and breast cancer (Prinz-Langenohl et al. 2001). Consistent with a role in DNA repair, chromosome breaks and centrosome abnormalities have been observed in patients deficient in folate (Heath 1966; Chen et al. 1989). In vitro, DNA strand breakage and uracil misincorporation increased in a time and concentration dependent manner after human lymphocytes were cultured with decreasing amounts of folate (Duthie and Hawdon 1998). DNA breaks are associated with an increased risk of cancer in humans. Moreover, folate deficiency impairs DNA excision repair in rat colonic mucosa (Choi et al. 1998). These data indicate that folic acid deficiency affects the stability of cellular DNA at the chromosomal and molecular levels (Choi and Mason 2000). While folate deficiency has been extensively documented by analysis of human plasma, folate status within skin has not been widely investigated. Nevertheless, the inefficiency of delivery of nutrients to skin argues that documented folate deficiencies will extend to skin. Additionally, photolysis of folate appears likely to deplete this nutrient in sun-exposed skin (Jablonski and Chaplin 2000). It has been reported that fair-skinned patients undergoing photochemotherapy for dermatological conditions have low serum folate concentrations, suggesting that folate depletion may occur in vivo (Branda and Eaton 1978). With regard to delivery, folate per se is too hydrophilic for effective topical skin delivery.

## 10.4 Strategy 3: Preventing Photoimmune Suppression

While the mechanisms leading to photoimmune suppression are still poorly understood, DNA damage is a major factor leading to reduced immune surveillance (Ullrich 2002). Two major cell targets are Langerhans cells where DNA damage leads to suppression of cell migration and antigen presentation functions and keratinocytes where DNA damage results in altered cytokine signaling and reduced immune function potentially involved in generation of T suppressor cells (Elmets et al. 1983; Cruz et al. 1990). In addition to DNA, two other targets have been identified for immune suppression. The photoconversion of urocanic acid to *cis*-urocanic acid has been implicated in immune suppression, although the molecular mechanisms are still poorly defined (Moodycliffe et al. 1993). Also, ROS generation including membrane lipid peroxidation has been implicated in immune

suppression (Ullrich 2002), possibly by altering signaling pathways at the membrane level, although DNA as the ultimate target is still a possibility. In view of the strong link between DNA damage and immune suppression, it is not surprising that agents that prevent DNA damage or enhance DNA repair reduce suppression (Gensler et al. 1999; Ullrich 2002). Additionally, several other agents offer promise for prevention of immune suppression at stages following DNA damage.

*Plant polysaccharides*. A number of plant polysaccharides, such as those found in *Aloe barbadenis*, appear to prevent immune suppression by mechanisms distinct from those that do so by preventing DNA damage (Strickland 2001).

*COX inhibitors*. Drugs that block production of PGE2 by cyclooxygenase activity have been shown to reduce photoimmune suppression, suggesting a role of overproduction of prostaglandins in immune suppression (Shreedhar et al. 1998).

*Retinoic acid*. Defective dendritic cell function caused by abnormal differentiation of these cells is an important mechanism of tumor escape from immune system control. All-*trans*-retinoic acid has been shown to induce maturation of these cells in cancer patients and this may suggest a role in modulating immune suppression (Almand et al. 2001).

*Niacin*. As described above, niacin has been shown to prevent photoimmune suppression (Gensler et al. 1999). While the ability of niacin to prevent immune suppression may be due to its ability to limit DNA damage by enhancing DNA repair, it should be noted that niacin has recently been discovered to stimulate the release of leptin (Kim 2002). Leptin is emerging as a hormone that modulates numerous protective effects in skin including immune modulation. Thus, it is possible that niacin prevents immune suppression via effects on leptin secretion.

## 10.5 Strategy 4: Enhancing the Epidermal Barrier

The epidermis of skin is a constantly renewing tissue. This renewal involves a complex series of events that involves proliferation of keratinocytes in the basal layer followed by terminal differentiation that ultimately leads to an epidermal barrier whose integrity is crucial to the protection of the organism from environmental insults. Several points need to be considered with regard to micronutrients in epidermal turnover. First, there is a growing body of evidence indicating that a significant percentage of the American population is deficient in a number of micronutrients and the constant turnover of the epidermis makes this tissue particularly vulnerable to micronutrient depletion. While there is limited data on micronutrient content of skin, studies have demonstrated that micronutrient deficiencies observed in plasma also are observed in skin (Peng et al. 1993), a wide range of tissue NAD content has been observed in human skin (Jacobson et al. 1999), and solar exposure has been demonstrated to deplete micronutrients (Jablonski and Chaplin 2000; Liebler and Burr 2000). Thus, skin is a likely site of micronutrient deficiencies with potentially adverse consequences leading to skin damage. Second, the constant renewal of the epidermal compartment places an important energy requirement on the organism. Thus, the nutritional status of micronutrients whose bioactive

forms play important roles in cellular energy generation is important to the integrity of the epidermal barrier (Jacobson et al. 2001). Micronutrients in this category include lipoate, niacin, ubiquinone, creatine, and carnitine. Third, the non-vascular nature of the epidermal compartment makes micronutrient delivery to this compartment inherently inefficient. The above considerations have led to the proposal that optimal energy metabolism will strengthen integrity of the epidermal barrier which in turn can lead to a decrease in skin cancer. Studies have shown that cell populations with altered growth properties within actinic keratosis lesions can be recognized by immune surveillance and removed. Alternatively, cell populations within such lesions can progress to cell populations (carcinoma in situ) that secrete proteases and other factors that allow escape from the epidermis. Thus, the status of the epidermal barrier integrity can be a deciding factor between the ultimate fates of removal or escape of abnormal cell populations from the epidermal compartment.

## 10.6 Innovative Agents for Skin Cancer Prevention are Needed

Several approaches have been taken to reduce the rate of skin damage and photodamage. Reducing the amount of damage that reaches critical biomolecules in the skin is the objective of sunscreens, antioxidants, and quenchers of photoexcited states (Fig. 2). Sunscreens aim to directly absorb sunlight photons and thus lessen the amount of damage that reaches the skin, and quenchers are designed to deactivate excited state molecules in skin prior to interaction with oxygen to limit the amount of ROS formed. Glycation inhibitors also are designed to limit ROS generation from solar irradiation of AGE-pigments in skin. Alternatively, antioxidants, which include Vitamins C and E as active ingredients, are designed to intercept damage to the skin by capture of ROS generated by sunlight exposure. While the approaches designed to reduce damage to the skin are beneficial, they have inherent limitations, as neither sunscreens nor antioxidants can effectively eliminate oxidative stress. Sunscreens absorb only a portion of the rays of sunlight that cause damage, many are not photostable for more than a few minutes in sunlight, and the feasible levels of antioxidants in skin creams can only partially reduce oxidative stress. Another approach to treating skin deterioration involves accelerating the removal of the upper layers of damaged skin to allow replacement with undamaged skin. The application of retinoic acid (tretinoin, Renova and Retin A) results in an increased rate of cell turnover allowing new cells to mature and replace damaged cells, but a side effect is a weakened skin barrier and increased photosensitivity. Chemical peels using agents such as alpha-hydroxy acids or beta-hydroxy acids cause a chemical exfoliation of the top layers of skin, again allowing new cells to replace the damaged cells that have been removed. Topical formulations of 5-fluorouracil represent an aggressive therapy for removal of skin lesions and a topical formulation of the cyclooxygenase (COX) inhibitor diclofenac has been approved for treatment of actinic keratosis. Surgical or laser procedures also can remove damaged skin. While these approaches play important roles in the treatment of skin damage, the high irritation potential, increased sunlight sensitivity, and long downtime for patient recovery from facial

disfiguration of most current treatments combined with the enormity of the problem indicates that new approaches to treat and prevent skin damage still are needed.

Lipophilic ester prodrugs have been used to increase the permeability of polar compounds for transdermal systemic drug delivery. With regard to micronutrients, some derivatives of Vitamins C and E have been prepared and used in skin care products, but a systematic, scientific base for rational development of compounds and their evaluation demonstrating optimal delivery of protective agents to the cellular components of skin has not been reported. For example, the realization that Vitamin E is more stable as an ester derivative resulted in the design of a compound that was less efficacious than the parent compound, but to our knowledge the flux rates into skin of Vitamin E esters have not been studied (Alberts et al. 1996). We predict that a more polar derivative of Vitamin E also would stabilize the compound and improve delivery. This approach to optimize skin micronutrients and/or chemoprevent genotoxic stress in skin with multiple agents is complementary to and integrative with existing approaches and new developments such as designing more effective sunscreens to limit skin damage.

## 10.7 Topical Delivery: The Cornerstone of a Skin Damage Prevention Strategy

We have reviewed above evidence indicating that several agents are therapeutic candidates for skin damage prevention. However, a major challenge for the development of prevention strategies for skin damage relates to the difficulty of delivering small molecules to skin. Delivery to skin via the blood circulation of nutrients taken orally is inherently inefficient as delivery is distal to other organs and numerous cell targets for skin cancer prevention are located in the epidermis which is non-vascular. The challenging in delivering many micronutrients topically is that they are small molecules that do not have optimal properties to insure prolonged skin residence time required for efficacy. For example, niacin, ascorbate, lipoate, creatine, carnitine, and folate are too polar for effective delivery while Vitamin E, Vitamin E acetate, and ubiquinone are too lipophilic. To resolve this problem, we have studied niacin (nicotinic acid) as a model nutrient to determine the feasibility of optimizing topical delivery to skin cells. Briefly, the strategy is as follows. Prodrugs designed for optimal delivery are synthesized as esters or thioesters of the parent micronutrient or drug. Once delivered to the epidermis, the abundant and non-specific esterases present there rapidly cleave the prodrugs back to the parent compound. The delivery properties are designed to provide a slow, continuous supply of micronutrient to skin cells to allow increased uptake by the cells. This strategy takes into consideration two distinct barriers that influence the delivery of small molecules to skin, lipophilicity of the stratum corneum and skin metabolic activity. Our formulation strategy controls the rate of partitioning of the prodrug in and out of the stratum corneum by designing derivatives with an optimal lipophilicity for such partitioning. Figure 4 shows a multiple compartment model that serves as the framework for the development of this delivery strategy. Briefly described below are features of the topical delivery strategy that have emerged from our research.

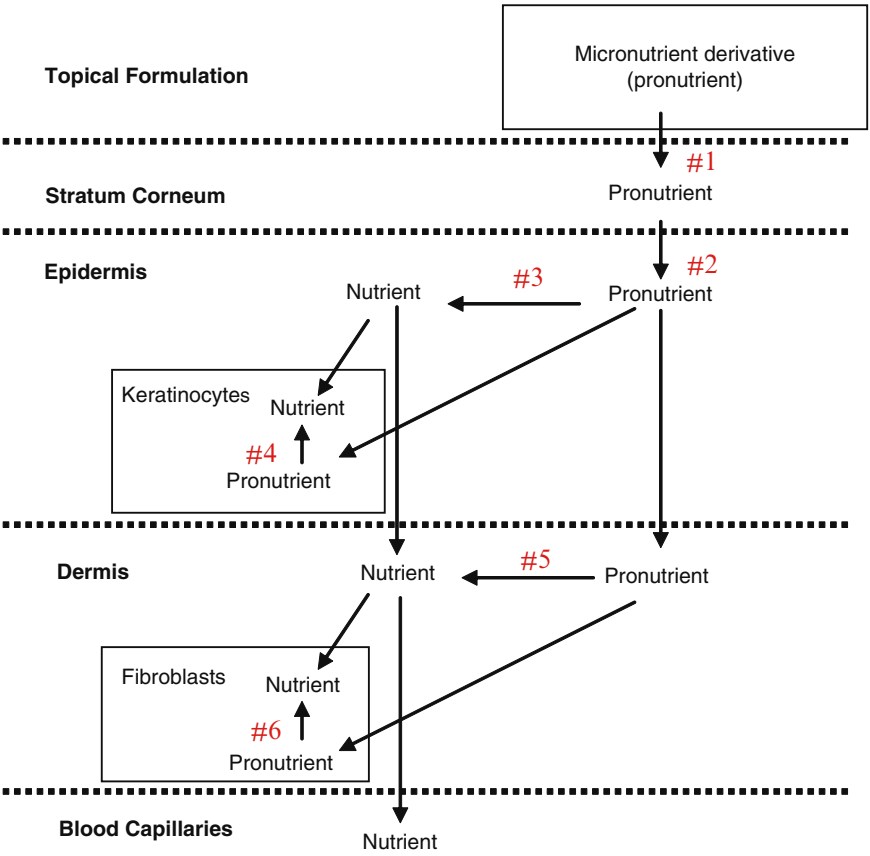


**Fig. 4** Multiple compartment model for topical delivery

*A pronutrient must effectively partition from the topical formulation into the stratum corneum*. The highly lipophilic nature of the stratum corneum dictates that a pronutrient be sufficiently lipophilic to effectively partition into the stratum corneum from the donor compartment, which can be a skin cream or lotion (arrow #1 in Fig. 4). As described in more detail below, the required lipophilicity needed for diffusion from the stratum corneum into the epidermis predicts that an efficacious pronutrient should be sufficiently lipophilic to rapidly partition from the cream or lotion into the stratum corneum. We have synthesized esters of nicotinic acid that are lipophilic derivatives that allow rapid diffusion from the topical formulation into the stratum corneum.

*The pronutrient must be stable in the topical formulation*. The lipophilicity of a prodrug should allow it to be formulated in a skin cream or lotion and the linkage of the nutrient derivative must be very stable in these formulations. We have shown that the prodrug lipophilicity optimal for delivery is such that the prodrug is easy to formulate in a cream or lotion. Also, in the case of niacin prodrugs, our developmental research examined

and identified compounds that were stable to chemical hydrolysis when formulated in a cream or lotion.

The pronutrient must partition from the stratum corneum into the epidermis at an optimal rate to achieve effective delivery to the cellular components of skin. (#2 in Fig. 4). Studies of drug structure-penetration relationships have provided useful information concerning partitioning from the stratum corneum to the epidermis (Tsai et al. 1992; Potts and Guy 1993; Webber et al. 1994). This rate of flux is controlled by a diffusion constant and for small uncharged molecules lipophilicity is the major factor that determines the diffusion constant. A correlation between skin permeability ($P_B$) and the physicochemical properties of the drug, such as octanol/water partition coefficient ($P_{oct/w}$) have proven to be of great value in predicting drug transport across skin. Figure 5 illustrates the relationship between compound lipophilicity, rate of flux from stratum corneum, and skin residence time (Roberts et al. 1978; Anderson et al. 1988).

A series of niacin esters were synthesized and their log $P_{oct/w}$ values were determined. The values demonstrated a linear relationship between alkyl chain length of the niacin ester and the logarithm of the octanol/water partition coefficient. These data allowed us to relate prodrug lipophilicity to niacin delivery and thus allowed identification of the lipophilicity range that provides an optimal rate of prodrug and thus drug delivery.

*The pronutrient must be efficiently bioconverted to active nutrient in skin*. The delivery approach that we designed for niacin involved the bioconversion of the pronutrient to niacin by the action of skin esterases. Studies on the esterase distribution of skin have shown that the stratum corneum has little or no esterase activity, the epidermis has the highest activity and the dermis has reduced activity relative to the epidermis (Sugibayashi et al. 1999). Delivery should be possible whether the bioconversion is extracellular (#3 and 5, Fig. 4) or following uptake by the target cells (#4 and 6, Fig. 4) since cells contain specific transport

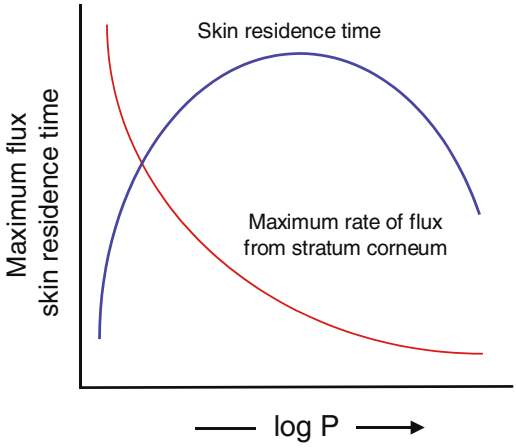


**Fig. 5** Relationship between compound lipophilicity, rate of flux from stratum corneum, and skin residence time

systems for niacin and the lipophilicity of the prodrug should make it readily bioavailable through passive diffusion also. Skin cells also contain esterases. Bioconversion was confirmed by experiments that determined the effect of niacin prodrugs on the content of the bioactive form of niacin, NAD, in skin cells. Thus, we have measured bioconversion of the prodrug to nutrient and then bioconversion of the nutrient to the active form of the vitamin in this case. In this manner, we were able to relate the major factor determining the rate of partitioning from stratum corneum to epidermis (prodrug lipophilicity) to the effectiveness of cellular delivery (skin cell NAD content).

## 10.8 Developing a Niacin Prodrug as a Potential Skin Cancer Prevention Agent

*Rationale*. Nicotinic acid was selected for development as the first topical agent in a series of micronutrients based on its known effects in preclinical studies. The diagram in Fig. 6 outlines the three predominant mechanisms of action of this compound. First, it has long been appreciated that nicotinic acid, as well as nicotinamide, can serve as the vitamin precursor of NAD and recent studies have demonstrated that NAD deficiency can occur in Western populations (Jacobson et al. 1995) and while the degree of deficiency may not reach that which elicits symptoms of pellagra, it may be relevant in the development of chronic disease states such as cancer. NAD is essential in energy metabolism and may be a very important factor in the continual epidermal renewal of skin, which is known to turnover approximately every 28–30 days. In addition, numerous

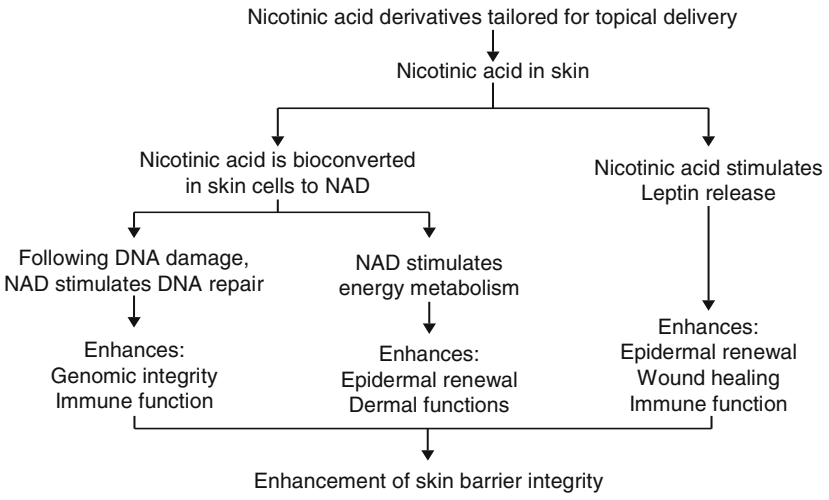
Nicotinic acid derivatives tailored for topical delivery
Nicotinic acid in skin
Nicotinic acid is bioconverted in skin cells to NAD
Nicotinic acid stimulates Leptin release
Following DNA damage, NAD stimulates DNA repair
NAD stimulates energy metabolism
Enhances: Genomic integrity Immune function
Enhances: Epidermal renewal Dermal functions
Enhances: Epidermal renewal Wound healing Immune function
Enhancement of skin barrier integrity

**Fig. 6** Known effects of nicotinic acid in skin

dermal functions are energy demanding. Since skin is the largest organ of the body, maintenance of optimal NAD for energy in skin by dietary means could be challenging, particularly during aging and following photodamage. Secondly, NAD serves as a substrate for the enzyme PARP-1, which plays an essential role in maintenance of genomic integrity and is rapidly consumed during genomic stresses such as UV radiation and environmental insults to skin (Jacobson et al. 1983). This pathway may also be important in immune function, which is critical following UV exposure. Thirdly, we have shown that nicotinic acid stimulates the release of the cytokine, leptin (Kim et al. 2002a), which has been shown to function in epidermal renewal, wound healing, immune function, and inhibition of tumor promotion. Based on these findings, a development program for a topical prodrug of nicotinic acid was initiated.

*Synthesis and characterization of niacin prodrugs*. The feasibility of developing a topical delivery system for skin protective agents was established by demonstrating that delivery of a niacin prodrug is controlled primarily by the rate of diffusion (lipophilicity). We synthesized, purified, and characterized niacin derivatives using alcohols varying in alkyl chain lengths from 8 to 18 carbon atoms to construct niacin derivatives.

To assess targeted delivery to skin, niacin derivatives were formulated in a compatible lotion and administered to the backs of hairless mice, once daily for 3 days. Skin samples from the site of application were evaluated for intracellular NAD content. This measurement assesses the net effect of diffusion of the prodrug through the stratum corneum to the cellular layers of skin, bioconversion to niacin, uptake by cells, and subsequent conversion to NAD. From Fig. 7, it can be seen that derivatives having log P values ranging from around 6 to 10 were effective at targeting delivery to skin cells with tetradecyl nicotinate (TN or Nia-114) (Log $P_{oct/w}$ of 7.5) most effectively elevating NAD in mouse skin at

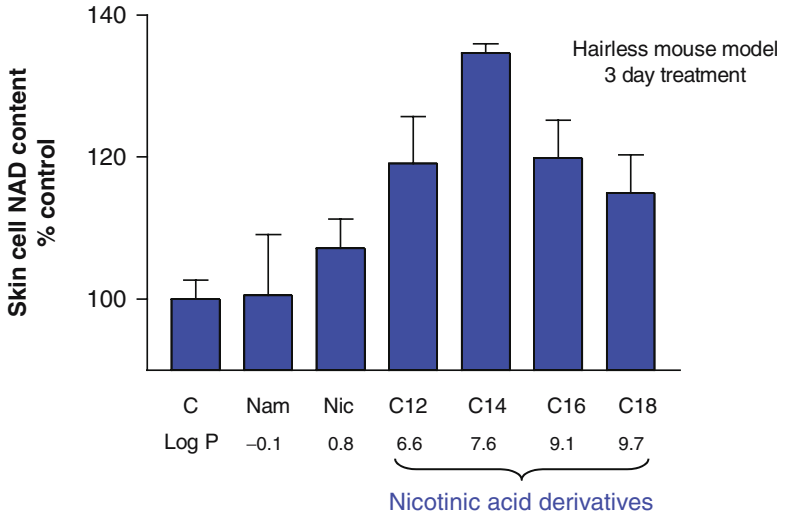


**Fig. 7** Effect of niacin derivatives applied topically on skin cell NAD

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the site of application. Also, it can be seen in Fig. 7 that the free forms of the vitamin, nicotinamide and nicotinic acid did not effectively deliver to skin cells to increase skin cell NAD content.

The proposed topical delivery system was designed to target small molecules to skin with minimal systemic exposure. To evaluate this effect, skin samples taken from the abdominal area (distal to the application site) were compared to that taken from the back (site of application). Minimal changes in NAD occurred in abdominal samples while significant increases were observed in the samples from the back as shown in Fig. 7. These data provide evidence for preferential delivery to the targeted tissue. These data show that tetradecyl nicotinate delivers niacin at a slow sustained rate at the site of delivery on the stratum corneum, allowing hydrolysis of the prodrug at a rate suitable for efficient uptake and bioconversion by skin cells. In contrast, lauryl nicotinate increased skin NAD content at both the site of application and at a distal site (data not shown), indicating tissue saturation at remote sites due to transdermal delivery. Using this lead candidate, tetradecyl nicotinate, dose- and time-response studies were carried out to determine the dosing concentration and schedule for optimal delivery to skin cells (Figs 8 and 9). Using 7 days as

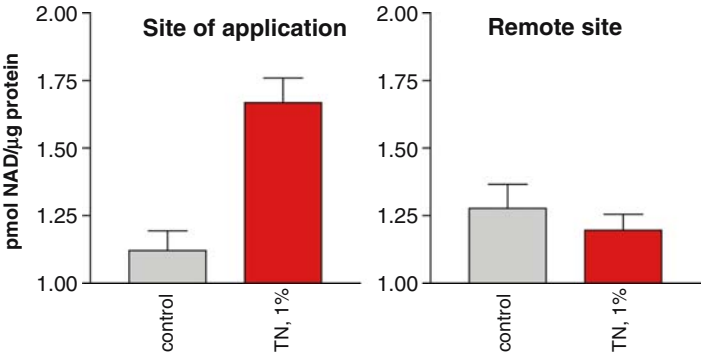


**Fig. 8** Preferential dermal delivery by tetradecyl nicotinate in mouse skin

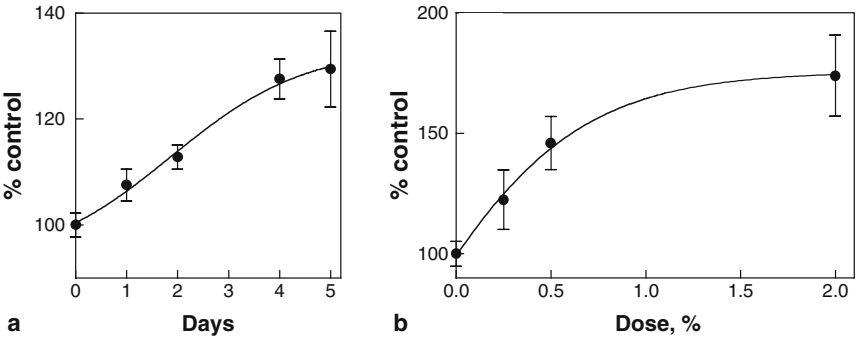


**Fig. 9** Dose (**a**) and time (**b**) effects of topical tetradecyl nicotinate treatment on NAD content of hairless mouse skin

an end point, concentrations of tetradecyl nicotinate up to 1.0% increased NAD in skin. The time course of skin NAD content is shown for 1% tetradecyl nicotinate. A plateau was observed at about 5 days.

Tetradecyl nicotinate was found to be stable at room temperature and at elevated temperatures for extended periods of time appropriate to further development. In addition, bioconversion studies showed that it was readily converted to nicotinate and the free alcohol, demonstrating that tetradecyl nicotinate functions effectively as a prodrug of niacin for topical delivery.

## 10.9 Clinical Development of Tetradecyl Nicotinate (Nia-114)

*Clinical evaluation of Nia-114*. This compound has undergone extensive safety evaluation in vitro and in vivo to determine the repetitive epidermal contact potential of a test material to induce primary or cumulative irritation and/or contact sensitization. The test material at 5% showed no potential for dermal irritation or allergic contact sensitization. Skin creams containing this compound are extremely well tolerated with daily use. No irritation was reported by study subjects or detected by study physicians. Measurements designed to detect even minimal irritation as skin redness showed a trend away from redness for Nia-114 treated skin. These data demonstrate that the controlled delivery of niacin using the prodrug srategy eliminates the vasodilation effects that occur when niacin is applied topically or taken orally. The prodrug strategy was designed to provide slow continuous delivery where the concentration of niacin reaching the circulation would be below the threshold to induce vasodilation. With these safety and tolerability evaluations completed, the clinical effects on skin of Nia-114 were then evaluated in multiple studies that have used a double blinded, placebo-controlled study design. Results of these studies are summarized below.

*Nia-114 simultaneously increases skin cell turnover and skin barrier integrity*. The effects of Nia-114 on stratum corneum turnover was measured by disappearance of Dansyl staining as a surrogate measure of the rate of skin cell turnover. Treatment with Nia-114 resulted in a highly statistically significant stimulation of skin cell turnover in the range of 7–11% compared to placebo. Thus, it is similar to other treatments for photodamage in its property to increase skin cell turnover; however, the magnitude of the effect is less than that observed for other treatments. The advantage of this approach to stimulate skin cell turnover is that the turnover occurs in a manner that strengthens skin barrier integrity as described below while other treatments that stimulate turnover do so to such a degree that skin barrier integrity is seriously compromised.

The effect of Nia-114 on the integrity of the skin barrier has been determined in a number of different ways that include determination of the rates of transepidermal water loss (TEWL), TEWL following a standard regimen of stratum corneum removal by tape stripping to assess effects on the upper epidermis, and by stratum corneum conductance determinations. Each of these methods has shown that Nia-114 strengthens the integrity of the skin barrier. The relationship between rates of TEWL and skin barrier integrity

has been validated showing that decreased rates clearly reflect a more intact skin barrier (Reeve et al. 1998). Nia-114 treatment resulted in a highly statistically significant ($p = 0.006$ versus placebo) decrease in the rate of TEWL of nearly 20% above the effect of the placebo alone. The effect of Nia-114 on skin barrier integrity has been assessed following removal of the stratum corneum layer of skin using a standardized protocol of tape stripping. Nia-114 treated arms showed a 20% decrease in the rate of TEWL at 18 weeks of treatment ($p = 0.07$ versus placebo). Stratum corneum conductance measurements showed a highly statistically significant progressive increase in skin barrier integrity for Nia-114 treated skin compared to placebo of 10% at weeks 12 ($p = 0.05$) and 18 ($p = 0.01$). The unique ability of Nia-114 to simultaneously stimulate skin cell turnover and strengthen skin barrier integrity is consistent with the known roles of nicotinic acid (Fig. 6). It will be interesting to determine whether strengthening the integrity of the epidermal barrier will limit progression of in situ cancers to metastatic cancers. Further studies will be needed to verify this hypothesis.

*Nia-114 dramatically increases skin barrier integrity in compromised skin*. Figure 10 shows the results of skin barrier assessment with placebo and Nia-114 treatment at 4 and 8 weeks in a group of atopic study subjects measuring rates of transepidermal water loss (TEWL) to assess barrier integrity. Research has shown that rates of TEWL are strongly correlated with severity of clinical symptoms in atopic subjects (Chamlin et al. 2002). The results show that the placebo had no significant effect on barrier function while the Nia-114 treatment resulted in an approximately 35 and 45% increase in barrier function at 4 and 8 weeks, respectively. This effect of Nia-114 on skin barrier integrity in atopic subjects has exciting implications for clinical evaluation of benefits of this compound (Chamlin et al. 2002). Furthermore, it has been reported recently that low levels of leptin are strongly correlated with the risk of atopic skin, which is of interest with regard to the leptin releasing property of nicotinic acid (Jacobson et al. 2002) and the role of leptin release in preventing skin tumor promotion (Thuillier et al. 2000). The data of Fig. 11 show examples of histological analyses of skin punch biopsies from a clinical trial evaluating the effects of Nia-114 on skin. The increase in layers of corneocytes of the stratum corneum, responsible for barrier function, observed over 12 weeks of treatment as compared to the placebo is remarkable and correlates with effects measured by physical methods.

*Nia-114 confers photoprotection*. The effect of Nia-114 treatment on the minimum time of UV exposure to cause erythema also was determined in two separate sets of experiments. Nia-114 treatment results in a photoprotective effect of approximately 9% relative to control (data not shown). Erythema following UV exposure results from DNA damage, and the increased skin resilience following treatment is consistent with the known effects of nicotinic acid on enhancement of DNA repair and strengthening skin barrier integrity. The photoprotective effect of Nia-114 treatment contrasts sharply with other treatments for photodamage where photosensitivity is often observed.

*Development of Nia-114 as a skin cancer prevention agent*. Preclinical data has generated a body of evidence that has led to a RAPID Award from the National Cancer Institute of the National Institutes of Health for development of Nia-114 as a skin cancer prevention agent. The RAPID program has supported preclinical toxicology and pharmacology studies. Based on additional data, (Jacobson et al. 2007a,b) it is now clear that the combined mechanisms by which Nia-114 is modulating skin responses are driving differentiation

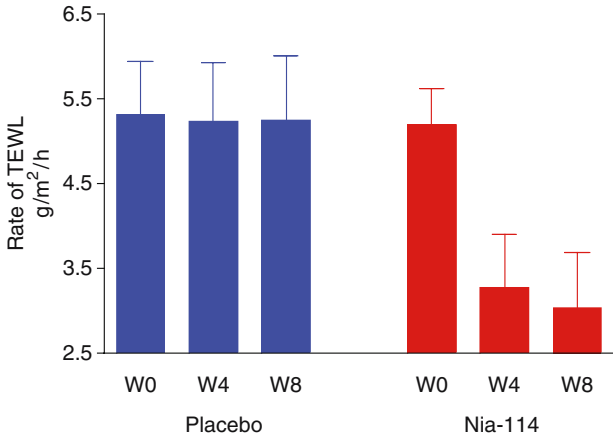


**Fig. 10** Decrease in rate of transepidermal water loss during treatment with Nia-114 in individuals with atopic skin

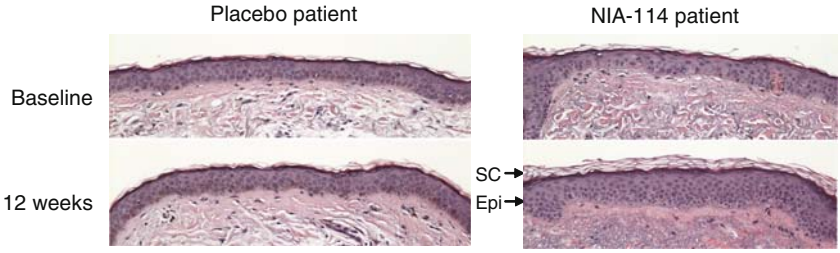


**Fig. 11** Histological analyses of Nia-114 effects on skin

of the epidermis. This finding has led to the filing an Investigational New Drug Application (IND) with the US Food and Drug Administration and execution of a Phase I clinical evaluation.

## 10.10 Summary

The complexity of processes that lead to skin damage are such that successful skin prevention strategies almost certainly will require a combination of agents that can provide prevention benefit by impacting different aspects of skin damage. Effective topical delivery of protective agents provides a solution to the difficulty of delivering to skin compounds taken orally. Topical delivery allows prevention to be targeted to the sites of damage, namely sun exposed skin, while minimizing systemic exposure. An increasing body of evidence

indicates that key micronutrients can combat skin damage by multiple mechanisms including the reduction of genotoxic stress that is clearly a major factor in accumulated skin damage. A critical factor in any prevention strategy must be the safety of the prevention agent since long-term human exposure will be required. The tolerance and safety of micronutrients makes them excellent prevention candidates. The known protective effects of nicotinic acid summarized in Fig. 6 have led us to develop a topical prodrug of this micronutrient and to begin development of this agent for skin cancer prevention. The data presented in this chapter indicate that the approach we have initiated allows effective delivery and provides benefit to skin with the potential to serve as a component of skin cancer prevention strategies. The approach is applicable to numerous other micronutrients and small molecule agents that have potential for skin cancer prevention

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# Skin Cancer Prevention

# 11

M. Lluria-Prevatt, D.S. Alberts

## 11.1 Epidemiology of Skin Cancer

Skin cancer is the most common malignancy in the world. One out of three new cancers is a skin cancer (Diepgen and Mahler 2002). More than one million cases of non-melanoma skin cancer (NMSC) (basal cell carcinoma [BCC] and squamous cell cancers [SCC]) occur annually (Jemal et al. 2008). Approximately 800,000 of these cancers are BCC and about 200,000 are SCC (Diepgen and Mahler, 2002). In Australia, NMSC accounts for 75% of all cancers and is 30 times more prevalent than lung cancer among men and ten times more prevalent than breast cancer among women (Burton 2000). Incidence rates for NMSC are increasing. An average increase of 3–8% per year since the 1960s has occurred in the white populations of Europe, the United States (US), Canada and Australia (Glass and Hoover 1989; Green 1992). Incidence data for NMSC are sparse because traditional cancer registries do not track NMSC, however it has been estimated that the incidence of NMSC is 18–20 times greater than that of melanoma. Incidence rates of NMSC increase proportionally with the proximity to the equator, with high cumulative Ultraviolet radiation (UVR) light exposure and with age (Diepgen and Mahler 2002). The incidence of NMSC has until most recently affected the older population – especially men who have worked outdoors, however the age of onset has steadily decreased. While the incidence rates for non-melanoma skin cancers continues to rise the mortality rate has decreased in recent years however there continues to be a substantial impact on morbidity, health and health care costs. In 2001, approximately 2,000 deaths were reported due to NMSC mostly due to metastasis of SCC to the lymph nodes and other sites. Early diagnosis and appropriate therapy result in a 95% cure rate. Prevention is the key management tool for NMSC.

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Melanoma is the skin cancer with the most fatal potential. Worldwide the number of melanoma cases is increasing faster than any other cancer. In 2008, an estimated 62,480 persons are expected to be diagnosed with melanoma and 8,420 deaths will occur due to melanoma (Jemal et al. 2008). Data from the US Surveillance, Epidemiology and End Results (SEER) registry demonstrated that melanoma was the most rapidly increasing malignancy in both sexes in the US from 1973 through 1997 (Lens and Dawes 2004). In the last few decades, the incidence rate of melanoma has substantially increased especially among the Caucasian population. During the 1970s, the incidence rate of melanoma was approximately 6% a year. However, the rate of increase has slowed to less than 3% per year. Statistics obtained between 1990 and 2003 demonstrated that the death rates for melanoma was one of four major cancers which were still increasing in males with only a 4.4% decrease in females (Jemal 2007). The rate of melanoma incidence is ten times higher in whites than in African Americans (Jemal et al. 2004). In Australia, melanoma is the fourth most common cancer among males and the third most common among females. Statistical data suggests that the lifetime risk for melanoma in Australia is now one in 25 for men and one in 34 for women (Burton 2000). In the US the lifetime risk of developing melanoma was 1 in 1,500 individuals in the year 1935 while in 2002, the risk was one in 68 individuals (Rigel 2002) and in 2008, the estimated risk is one in 41 for males and one in 61 for females (Jemal et al. 2008). It has been estimated that melanoma is the sixth most common cancer among males and the seventh most common cancer among females in the US in the year 2008 (Jemal et al. 2008).

Non-melanoma skin cancer (NMSC) arises from keratinocytes and originates in the epidermis. BCC originates from the basal cells of the epidermis and occasionally those of the infundibular and outer root sheath of the hair follicles (Lang and Maize 1991). BCCs are rarely fatal and seldom metastasize however they can be locally invasive and destructive (Randle 1996). SCC originates from keratinizing cells of the epidermis. These tumors are more aggressive than BCC and more likely to metastasize. While death rates remain low for NMSC, the incidence is very high and therefore treatment is very costly. The US Medicare system cost of treating NMSC between 1992 and 1995 was nearly $500 million consuming 4.5% of all Medicare cancer costs (Housman et al. 2003). Of additional concern, individuals who develop NMSC are at increased risk for the development of new skin cancers within the next few years following diagnosis (Diepgen and Mahler 2002). A follow-up study found that 52% of individuals diagnosed with SCC developed subsequent NMSC within 5 years of initial therapy (Frankel et al. 1992). Prevention of NMSC is a sensible strategy to lowering these costs.

Melanoma arises from melanocytes, the pigment producing cells, which reside in the dermal and epidermal junction of the skin. Survival is strongly associated with the thickness of the lesion. If caught early, melanoma can be cured by surgical excision of thinner lesions (0.75 mm or less) and has a 5-year survival rate of 99% (Koh 1991). Patients with thicker melanoma (more than 3.5 mm) have a steep decrease in survival rates (Lens and Dawes 2004). Tumors greater than 4 mm in depth are associated with a 5-year survival rate of less than 50%. If the disease spreads the survival of 5 years after diagnosis is only about 30–40%. Estimated annual cost for the treatment of melanoma in 1997 was $563 million in the US (Tsao et al. 1998). Stage I and II disease comprised 5% of the total, while stage II and stage IV disease consumed 34 and 55% of the total cost, respectively. Aggressive primary prevention could substantially reduce this economic burden.

## 11.2 Risk Factors

### 11.2.1 Ultraviolet Radiation Exposure

Aside from genetics, the major risk factors for all skin cancers are exposure to ultraviolet radiation (UVR) and skin color or the inability to tan. All skin cancers have been associated with exposure to sunlight however the pattern of sun exposure may vary between skin cancers types.

UVR is comprised of wavelengths from 200 to 400 nm. The ozone of the earth's atmosphere absorbs most light wavelengths below 290 nm. Therefore, UVB (290–320 nm) and UVA (320–400 nm) are the only portions reaching the earth's surface. UVR light reaching the earth's surface is comprised of 90–99% UVA and one to 10% UVB. UVR causes many biological reactions in the skin, including inflammatory response in a sunburn, hindrance of immune activity, premature aging and damage to DNA resulting in potential development of skin cancer (Dissanayake et al. 1993).

UVR exposure causes an increase in reactive oxygen species (ROS) which can overwhelm the natural antioxidant defense mechanisms in the skin. This oxidative stress can result in ROS that interact with proteins, lipids and DNA (Berton et al. 1997; Li et al.1996). UVR can result in mutations in genes that regulate cell proliferation and repair. UVR-induced linkage between two adjacent pyrimidines (cytosine or thymine) on the same DNA strand is usually repaired by nucleotide excision repair enzymes before replication. However, if the repair fails or is delayed fixed DNA mutations can occur when DNA polymerase inserts adenine dinucleotide (AA) opposite the unrepaired dimer. The erroneous pairing of AA with CC or CT linked photoproducts mutations are observed as CC→TT and C→T, respectively. These are characteristically induced by only UVR and as such are designated UVR signature mutations (Wikonkal and Brash 1999).

In the past, UVB was thought to be more important than UVA in the generation of sun damage and skin cancer. However, UVA has become increasingly suspect in the development of skin cancer (Runger 1999). The photocarcinogenesis of UVA differs from UVB in that UVA is not readily absorbed by DNA, but is absorbed by other molecules within the cell, giving rise to reactive oxygen species, which in turn damage DNA, membranes and other cellular constituents (de Gruijl 2000). UVA has been shown to induce mutations in DNA including p53, which is discussed later in this chapter as an important genetic marker for NMSC (Burren et al. 1998). UVA has been found to be an important factor in the development of melanoma. Several investigators have argued that UVA is more relevant in melanoma causation than the UVB range (Setlow et al. 1993). Much of the evidence of UVA exposure as a risk factor for melanoma has come from epidemiological studies of users of sunbeds or tanning equipment with spectral output that is in the UVA range (Swerdlow and Weinstock 1998; Wang et al. 2001). A study of women from Norway and Sweden found that the women who visited a tanning parlor at least once a month were 55% more likely to later develop melanoma than women who did not artificially suntan. Those who used sunlamps during their 20s had the greatest risk, approximately 150% higher than

similarly aged women who did not use tanning beds (Veierod et al. 2003). Further contributing to the controversy, a preclinical study showed that UVB, and not UVA, exposure promoted melanoma growth in a mouse model (De Fabo et al. 2004).

Chronic exposure to UVR is the predominant cause of NMSC. Over 80% of these cancers develop on parts of the body exposed to the sun including the face, neck and arms (Diepgen and Mahler 2002). Incidence rates for NMSC correspond well with increased UVR exposure as demonstrated by the increased incidence among individuals with occupational or recreational outdoor exposure or who reside at latitudes closer to the equator (Diepgen and Mahler 2002). Many studies have shown an inverse relationship between latitude and NMSC incidence (Almahroos and Kurban 2004). A report from Southeastern Arizona suggests the incidence rates of NMSC in Arizona are three to six times higher than those in subjects with similar skin type and living in regions of higher latitude (Harris et al. 2001a,b). A compilation of these and several other studies demonstrates a more than 50-fold difference in rates of NMSC incidence between Australia and Arizona (low latitude) and Finland (high latitude), with the higher incidence occurring in the lower latitudes (Almahroos and Kurban 2004). Several Australian studies have demonstrated that people in countries with high ambient solar radiation have a higher incidence of NMSC than migrants with the same genetic background from countries with lower ambient solar radiation (Almahroos and Kurban 2004). People who move during childhood to the countries of high ambient solar radiation from countries with low ambient solar radiation have equal incidence of NMSC as natives (English et al. 1998). However, individuals who make this same move later in life have a lower incidence. This data supports the idea that NMSC develops from a chronic exposure of UVR. The risk factor of skin color for the development of NMSC is demonstrated by the lower risk of ethnically darker skinned migrants.

Melanoma incidence is also associated with exposure to UVR. Childhood sunburns and intense intermittent sun exposure are major risk factors for melanoma (Gilchrest et al. 1999). Anatomic locations of melanoma development support the basis for intermittent UVR exposure as a risk factor. Melanoma is most commonly found on the trunk of men and the trunk and lower extremities of women. These sites are not normally acclimated to the sun by chronic exposure, but rather tend to be exposed during outdoor recreational activities. The effects of the sun on the development of melanoma are modulated by skin type. Light pigmentation increases the risk of the development of melanoma.

In a study conducted at the University of Arizona, risk factors for SCC were evaluated among 918 Arizona residents with sun-damaged skin (at least ten clinically assessable AK lesions) who had been randomized to the placebo arm of a skin cancer chemoprevention trial (Foote et al. 2001). As shown in Table 11.1, risk factors for BCC included older age, male gender, red hair color and at least 10 years' residence in the state of Arizona, which is located in a lower-latitude region of the US with documented rates of SCC and BCC that are among the highest in the world (Harris et al. 2001a,b).

### 11.2.2 Other Risk Factors

The presence of precancerous lesions increases the risk of developing skin cancer. For SCC, the precancerous lesion is actinic keratosis (AK) and for melanoma, the precancerous

lesions is thought to be dysplastic nevi (DN). BCC does not appear to have a precancerous lesion however the presence of AK can often be an indicator of risk. Additional risk for skin cancer includes genetics, immune suppressive disease, past history of skin cancer and occupational exposure to coal tar, pitch, creosote, arsenic compounds or radium. Age, male gender, and DNA repair disorders such as xeroderma pigmentosum are also risk factors for skin cancer. For melanoma, additional risk factors include one or more family members who had melanoma and a large number of moles (risk increases with number of moles) or the presence of DN. Approximately 5–12% of patients with melanoma have a family history of melanoma in one or more first-degree relatives (Goldstein and Tucker 2001). Mutations in two melanoma susceptibility genes, CDKN2A (p16) located on chromosome 9 (9p21) and CDK4 located on chromosome 12 (12q13) have been identified. Mutations in p16 have been identified in 20% of tested melanoma families (Bishop et al. 2002).

### 11.2.3 Genetic Alterations in NMSC

Genetic studies of AK and SCC have demonstrated alterations on possible tumor suppressor genes of chromosomes 9p, 13q, 17p, 17q and 3p (Quinn et al. 1994; Hunter 1997). The targets for most of these mutations have not been identified except for p53, which lies on chromosome 17p. p53 is a tumor suppressor gene that plays a role in protecting cells from DNA damage. Recognized genetic targets in NMSC include p53 mutations demonstrated in a progression of normal skin to sun damaged skin to AK to SCC (Einspahr et al. 1999). p53 mutations have also been identified in BCC (Matsumura et al. 1996). Many of these mutations are CC→TT or C→T changes at dipyrimidine sites suggestive of UVR damage (Tsao 2001).

Results from an analysis of genetic changes in 36 AKs and 23 invasive SCCs (Rehman et al. 1996) suggest that the relationship between the accumulation of genetic change and behavior for NMSC is complex. However, the overall pattern of autosome loss in AKs was similar to that seen for SCCs. Loss of chromosome 17p was the most frequent target of loss of heterozygosity (LOH), which is consistent with data showing a high rate of UVR-induced mutations in p53 (Brash et al. 1991), detection of p53 mutations in irradiated skin and cultured keratinocytes (Nakazawa et al. 1994), and evidence showing p53 mutations in pre-invasive lesions (Einspahr et al. 1999). However, the number of SCCs with chromosome 17p loss far exceeded the number in which mutations were detected in p53 exons 5–8, consistent with the presence of other targets of inactivation on chromosome 17 (Wales et al. 1995). Increased $p21^{WAF1/CIP1}$ immunostaining and p53 immunostaining were observed in 97% and 83% of AKs, respectively, and were observed in lesions without any detectable LOH or p53 mutation, suggesting that changes in proliferation, $p21^{WAF1/CIP1}$ expression, and p53 expression may precede allelic loss or p53 mutation. A large number of AKs showing multiple areas of LOH and p53 mutation may not have acquired the relevant genetic change to allow invasion of the underlying dermis (Wales et al. 1995).

Genetic alterations in NMSC also include mutations in the ras gene. The frequency of ras mutations in SCC ranges up to almost 50% and up to 30% in BCC (Pierceall et al. 1991). Mutations in ras have also been identified in AKs (Spencer et al. 1995). Different rates reported for SCC and BCC ras mutations may reflect different techniques, different study populations and/or the differing molecular epidemiology of low and high sun exposure.

Although more commonly reported in melanoma, recent studies suggest alterations in p16 can be found in up to 24% of SCC and 3.5% of BCC (Soufir et al. 1999). Several of the detected mutations were UVR signature mutations. These mutations may account for alterations observed on chromosome 9p21 in SCC (Tsao 2001).

Genetic alterations in BCC are found in both hereditary and sporadic cases. The PTCH gene is found in patients with nevoid BCC syndrome, characterized by the rapid development of numerous BCCs early in life (Tsao 2001). Recent studies have demonstrated that 15–39% of these patients harbor mutations in the PTCH gene (Aszterbaum et al. 1998).

### 11.2.4 Genetic Alterations in Melanoma

Linkage studies of families with multiple cases of melanoma have been important in pursuing genetic analysis however the genetic relationship between melanoma and the dysplastic nevus syndrome are complex. Karyotype studies of both familial and sporadic melanomas frequently showed large deletions of band region 1p36, del(1)(p36.1–p36.3) (Dracopoli et al. 1994), suggesting that multiple tumor suppressor genes in this region were deleted. The PITSLRE protein kinase gene locus maps to band region 1p36. Several of its products may affect apoptotic signaling (Lahti et al. 1995). Studies have demonstrated alterations in the PITSLRE protein kinase gene complex in melanomas (Wymer et al. 1997).

Another frequently altered chromosome region, 9p21, contains a group of genes involved in cell cycle regulation. Among several potential tumor suppressor genes located on 9p21, p16 (CDKN2A/p16$^{ink4a}$) is the most important melanoma susceptibility gene identified to date with germline mutations present in 9p-linked melanoma families (Hussussian et al. 1994; Kamb et al. 1994) and in 30–50% of members of melanoma kindreds (Halachmi and Gilchrest 2001). p16 inhibits the ability of cyclin dependent kinases, CDK-4 and CDK-6, to activate substrates needed for progression past G1 of the cell cycle and therefore acts as a cell cycle check point protein (Liggett and Sidransky 1998). Germline mutations in the gene encoding CDK4 have also been described in a small number of melanoma-prone cases (Zou et al. 1996; Soufir et al. 1998). In sporadic tumors, loss of p16 protein expression has been shown to occur only in invasive and metastatic stages of melanoma and to be infrequent in primary thick nodular melanoma (Reed et al. 1995; Straume and Akslen 1997). The loss of p16 also seems to be associated with recurrent disease and has been the most useful marker for progressive disease. Alterations in p16 include CpG island methylation and translation repression mutations in the five prime untranslated regions (Haluska and Hodi 1998). Trascriptional upregulation of p16 has been shown in melanoma cells following UVB irradiation (Piepkorn 2000). UV-induced mutations of p16 have been reported in epithelial skin tumors from sporadic patients and from xerderma pigmentosum patients, who suffer from hypersensitivity to UVR (Soufir et al. 2000).

The phosphatases PTEN/MMAC1, located at 10q23,3, have been found deleted in more than 40% of melanoma cell lines (Ortonne 2002). In a study of melanoma progression from normal skin, acquired melanocytic nevi and cutaneous melanoma, nuclear PTEN expression was lost in both benign and malignant melanocytic lesions. However, the benign tumor retained cytoplasmic expression while the cutaneous melanoma demonstrated a complete

lack in PTEN expression (Tsao et al. 2003). Bcl-2 has been demonstrated to be over expressed in melanoma cells (Jansen et al. 2000). In addition, approximately a 20 or 40% increase of Bcl-$X_L$ mRNA levels was detected in primary or metastasized melanoma tissue, respectively. The metastasized melanomas expressed higher Bcl-$X_L$ than their matched primary tumors. These studies also showed that the expression of Bcl-$X_L$ resulted in UVB resistance in both primary and metastatic melanoma cells (Zhang and Rosdahl 2006). The transcription factor AP-2, and three of its downstream targets, c-kit, E-cadherin and p21, were found to be involved in later phases of melanoma progression (Baldi et al. 2001).

Dr. Arbiser of Emory University School of Medicine identified the activation of mitogen-activated protein kinase (MAPK) as an early event in melanoma progression (Cohen et al. 2002). One hundred and thirty one melanocytic lesions, ranging from atypical nevi to metastatic melanoma, were studied for the expression of phosphorylated (active) MAPK and two target genes known to be induced by MAPK signaling, tissue factor and vascular endothelial growth factor. While MAPK activation was positive in only 21.5% of benign nevi (with mild atypia), MAPK activation was seen in both radial and vertical growth phase melanomas. These findings suggest MAPK signaling as a potential target of chemoprevention in early melanoma.

In one study, 5–35% of various graded melanomas or nevi had some type of ras mutation (Yasuda et al. 1989). Within the ras family, N-ras has the most significant association with melanoma progression. Herlyn and colleagues found a role for ras in approximately 15–20% of melanomas with a positive association with sun exposure (Herlyn and Satyamoorthy 1996). Developments from the Cancer Genome Project have revealed that 66% of melanomas tested had a mutation in BRAF with the same single substitution occurring in 80% of melanomas (Davies et al. 2002). BRAF is known to play a role in cell growth and division. Most recently, investigators observed this same BRAF mutation (resulting in the V599E amino-acid substitution) in 63 of 77 (82%) histologically diverse nevi including four of five (80%) dysplastic nevi (Pollock et al. 2003). This mutation was observed in 68% of melanoma metastases and 80% of primary melanoma. By introducing an activated mutation of BRAF into cultured melanocytes, investigators showed that BRAF can act as an oncogene in early stages of melanoma. This activation resulted in a constitutive activation of MEK and ERK and ultimately tumorigenicity in nude mice (Wellbrock et al. 2004). These findings demonstrate that the mutation of BRAF and activation of the RAS–RAF–MEK–ERK–MAP kinase pathway, which mediates cellular responses to growth signals, is a crucial and early step in the progression of melanoma.

## 11.3 Screening and Early Detection

Early screening of SCC is often done by the diagnosis of AK. Self exams are strongly recommended. Warning signs include a skin growth that increases in size or changes color or thickness or a sore that continues to crust, bleed or itch. Identification of these changes warrants a more extensive check up from a dermatologist. A precursor lesion to SCC, AK often requires treatment. AKs can be difficult to treat and require frequent visits to

a dermatologist, since patients usually have multiple AKs that present at different times. The treatment for AK is usually cryosurgery with liquid nitrogen, excision or topical 5-FU cream (International Medical News Group 2002). Cryosurgery is the most common treatment but is associated with blistering, scabbing, hypopigmentation, inflammation and occasionally pain. Treatment with 5-FU often results in severe blistering. Other options for AK treatment include dermabrasion or chemical peeling (Dinehart 2000). Topical diclofenac (Del Rosso 2003), imiquimod (Berman et al. 2004), and aminolevulinic acid (in photodynamic therapy) have also been approved by the US Food and Drug Administration (FDA) for the treatment of AK. While these drugs can be effective, they also cause painful and irritating local skin toxicities. Appropriate chemoprevention strategies of AK or pre-AK treatment would not only reduce incidence of SCC but also eradicate the need for these disagreeable treatments mentioned above.

Screening for early melanoma includes a dermatological assessment however self-examinations are strongly recommended as well. This assessment includes a review of one's moles carefully looking for what the Skin Cancer Foundation calls the ABCDs of melanoma: asymmetry, borders, color and diameter. Most early melanomas are asymmetrical where the common mole is round and symmetrical. Early melanomas often have irregular borders with scalloped or notched edges. Normal moles have smooth borders. The color of early melanoma tends to have several shades of brown, tan or black and as the melanoma progresses the colors red, white or blue may appear. Normal moles tend to be a single shade of color. Early melanomas tend to grow larger than normal moles with diameters of at least 6 mm. The discovery of any of these characteristics should be promptly reported to a physician preferably one that specializes in skin cancer and is trained to identify early signs of melanoma.

It is apparent with the increase of skin cancer incidence, incomplete resolution by early detection and the current treatments, there is an urgent need to develop well-tolerated and effective prevention strategies for NMSC and melanoma.

## 11.4 Prevention of Skin Cancer

### 11.4.1 Primary Prevention

Since exposure to UVR is a major risk factor in the development of skin cancer, the focus of primary prevention has been to limit exposure to UVR. The recommendation from the American Academy of Dermatology, the American College of Preventive Medicine and the American Cancer Society are: (1) to reduce sun exposure during peak hours of intense ultraviolet exposure (usually 10 a.m. to 4 p.m.); (2) to wear protective clothing to cover as much of the skin as possible, including long sleeved shirts and hats with wide brims; and (3) to seek shade (Manson et al. 2000). Public health campaigns have been underway since the 1980s for the prevention of skin cancers. These campaigns recommend limited exposure to sun, the use of sunscreen, and early detection through screening. In Australia,

where skin cancer is of epidemic proportions (two out of three people born in Australia will likely require treatment for at least one skin cancer in their lifetime) (Giles et al. 1988), major campaigns have taken place such as the "Slip! Slop! Slap!" ("slip" on a shirt, "slop" on sunscreen, "slap" on a hat), "Sunsmart," and "Me No Fry." While 90% of Australians now recognize the dangers of skin cancer and the associated risks (Borland et al. 1992; Hill et al. 1993), the relationship between education and incidence reduction is still unclear. In an article by a leading Australian academic dermatologist, several cohort studies are reviewed and indicate some leveling or reduction in skin cancer incidence in younger populations of Australia, potentially associated with the success of the skin cancer awareness public campaigns (Marks 1999).

The 2003 Cancer Progress Report from the US Department of Health and Human Services and related departments reports limited success in the US population's attitudes toward sun exposure (2001). This report contains data gathered by the Centers for Disease Control and Prevention/National Center for Health Statistics. In the year 2000, 60% of adults said they were likely to seek some sort of sun protection, 31% were likely to use sunscreen, 26% were likely to use sunscreen with a sun protection factor (SPF) of 15 or higher, 32% very likely to wear protective clothing and 28% were very likely to seek shade. This data shows an increase from 1998 where there was an actual decline in the sun protection from previous years. In the 3 June 2002 issue of the Philadelphia Inquirer, the author remarks "Most adolescents avoid sunscreen like a summer reading list" (Uhlman 2002). The desire for a golden tan and the messy inconvenience of sunscreen outweighs the distant threat of skin cancer in the minds of these adolescents. In a study of sun protection practices in adolescents, only one third of the respondents reported routine sunscreen use during the past summer (Geller et al. 2002). Eighty three percent reported sun burning at least once and 36% reported three or more burns during the previous summer. Nearly 10% of all respondents used tanning beds during the previous year, with 24.6% of girls age 15–18 reporting tanning bed use. Many girls who used tanning beds reported a belief that it was worth getting burned. These findings are very alarming considering that tanning in the teen years is a key factor for lifetime cumulative sun exposure and increased risk for skin cancer, particularly melanoma, which is clearly related to early age sunburns.

While advocated as an important protection against sun exposure, sunscreen use has created some controversy. Most sunscreen products may not offer adequate protection against the harmful effects of the sun's ultraviolet radiation. Protection provided by sunscreen is based on the SPF (sun protection factor); however, this is only indicative of a reduction in erythema. Therefore, individuals who apply sunscreen often expose themselves for longer periods of times in the sun because of the lack of uncomfortable effects due to erythema. As discussed earlier, UVA exposure is an important risk factor in development of skin cancer. Most sunscreens are inadequate in absorbing the longer wavelengths of the UVA portion of the sun's spectral output and, therefore, may only provide a portion of the protection needed for skin cancer prevention.

Observational studies of melanoma suggest that sunscreen use may be associated with increased risk, potentially due to fair-skinned individuals spending more time in the sun without the visible effects of sunburn (Autier et al. 1995). Other studies also have found that frequent use of sunscreens was associated with a relative risk of 1.8–2.8 for cutaneous melanoma (Klepp and Magnus 1979; Graham et al. 1985; Beitner et al. 1990). Consistent

with these epidemiologic studies, one study in mice treated with sunscreen demonstrated a lack of protection toward UVR-induced melanoma growth (Wolfe et al. 1994). In this study, C3H mice were transplanted with K1735 melanoma cells and exposed to UVR subsequent to sunscreen application. The sunscreen preparations contained o-PABA (octyl-*N*-dimethyl-*p*-aminobenzoate), 2-EHMC (2-ethylhexyl-*p*-methoxycinnamate) and BP-3 (benzophenone-3). This formulation was effective in reducing histopathologic alterations in the mouse ear skin but failed to prevent UVR-induced inflammation and melanoma growth.

### 11.4.2 Secondary Prevention

The current primary methods for skin cancer prevention, including behavioral modification and the use of sunscreens, have not proven sufficient to protect against rise in skin cancer incidence. Therefore, other strategies of prevention need to be coupled with primary prevention. The most promising of these strategies are the development of chemopreventive agents, which target early stage or pre-cancerous lesions. Sporn (Sporn and Suh 2000) describes chemoprevention as a "pharmacological approach to intervention in order to arrest or reverse the process of carcinogenesis." He emphasizes the importance of an increased cancer research effort to control carcinogenesis "rather than attempting to cure end-stage disease." Control of carcinogenesis should be targeted at early stages because "it is easier to fix anything when the smallest numbers of its components are broken." The control of carcinogenesis through chemoprevention has gained credibility due to the FDA approval of tamoxifen for reducing breast cancer (Fisher et al. 1998; Lippman and Brown 1999) and from FDA approvals of agents for treating intraepithelial neoplasias (IENs) such as diclofenac for AK (O'Shaughnessy et al. 2002) and celecoxib for familial adenomatous polyposis (Steinbach et al. 2000). Agents for chemoprevention are ultimately applied to the general healthy population at high risk for particular cancers. Safety and efficacy must be established in large-scale prospective randomized clinical trials. Furthermore, agents need to be non-toxic, inexpensive and available in oral or topical form (for skin). Clinical trials in patients with premalignant lesions are initially performed to investigate the modulation of biomarkers as surrogate endpoints. Lippman and Hong equate the current cancer chemoprevention studies to a delay in cancer development where the measures include a reduction in the rate of tumor development and overall decrease in the incidence of number of tumors (Lippman and Hong 2002). Meyskens described chemoprevention as an interaction between sciences of carcinogenesis, cellular biology and cancer screening/early detection and cancer prevention/treatment (Meyskens 1988). Clearly, all of these scientific disciplines are required to develop highly efficacious chemopreventive strategies for skin cancer. Several reviews have been written which describe the current development of chemoprevention of skin cancer (Bowden 2004; Stratton et al. 2000, 2005; Wright et al. 2006).

For skin cancer, the eradication of AK and DN would most likely reduce the incidence of NMSC and melanoma, respectively. The approach employed in the development of chemopreventive agents include: (1) availability of precancerous lesions (AK or DN) to evaluate the potential reduction in risk of progression; (2) identifying target molecules that are

often modified and subsequently contribute to skin carcinogenesis; (3) developing animal model systems to test potential chemopreventive agents in skin; (4) delivery of highly potent agents directly into the epidermis even more specifically through the development of prodrug formulations (discussed in Chap. 10), and (5) availability of intermediate molecular or histologic markers of the carcinogenic process to be used as endpoints.

### 11.4.3 Targeting Precursor Lesions for Chemoprevention

Current chemoprevention trials evaluate the efficacy of chemoprevention agents by the eradication or reduction of intraepithelial neoplasias (IENs). In skin, the IENs include AK for SCC and dysplastic nevi (DN) for melanoma. Individuals with AK are at increased risk for developing NMSC, and the presence of DN is the single most important risk factor for developing melanoma.

In general, IENs are near-obligate cancer precursor lesions that have genetic abnormalities, loss of cellular control function, similar phenotypic characteristics of invasive cancer and are risk markers for cancer. The presence of IENs in an individual is indicative of an increased likelihood of developing invasive cancer as compared to unaffected individuals (O'Shaughnessy et al. 2002). The American Association for Cancer Research (AACR) Task Force on the Treatment and Prevention of Intraepithelial Neoplasia recommends targeting individuals with or at risk for IENs for new agent development because of the potential preventive consequence on developing invasive cancer (O'Shaughnessy et al. 2002). IENs have been described for many types of cancers including: colorectal adenomas for colorectal cancer; dysplastic oral leukoplakia for head and neck cancers; Barrett's esophagus for esophageal cancer; cervical intraepithelial neoplasia for cervical cancer; prostatic intraepithelial neoplasia for prostate cancer; transitional cell carcinoma in situ for bladder cancer; and AK for NMSC.

Targeting precancerous lesions for chemoprevention is a rational strategy for the reduction of SCC incidence. Evidence for this rational includes: (1) the FDA approval of diclofenac for treating AK as a preventive measure against SCC (O'Shaughnessy et al. 2002) and (2) a report from the Southeastern Arizona Skin Cancer Registry that suggests the leveling of SCC incidence in southeastern Arizona could be due to the removal of the precursor lesion, AK, while BCC incidence appears to continue to rise because there is no known precursor lesion for BCC to be removed or treated (Harris et al. 2001a,b).

AK, also known as solar or senile keratoses, are cutaneous lesions with chromosomal abnormalities that occur primarily on sun-exposed skin surfaces (Callen 2000). AK is a proliferating mass of transformed neoplastic keratinocytes confined to the epidermis. AKs develop on the surface of the skin as thickened cornified, scaly lesions (O'Shaughnessy et al. 2002). Papules and plaques are often found on a background of sun-damaged skin with telangiectasias, hyper or blotchy pigmentation, and a yellowish hue. The lesions range in size from 1 to 2 mm papules to large plaques (Callen 2000). AKs are most often diagnosed by histopathologic examination, since diagnosis by appearance can often be unclear as to whether the lesion is an AK or SCC. Typical histologic characteristics of AKs include irregular arrangement of cells with atypical, pleomorphic keratinocytes at the basal

cell layer demonstrating nuclear pleomorphism, loss of polarity, crowding of nuclei and disordered maturation (Callen 2000).

The lack of significant cytological differences between AK and SCC gives rise to the premise that AKs represent early SCCs (Dinehart et al. 1997). Several investigators consider AKs to be precursors or early forms of SCC (Glogau 2000; Salasche 2000). Inasmuch as AK is well accepted as a precursor to SCC, the US Centers for Medicare and Medicaid Services have added a national coverage policy to include the treatment of AK (2002). The percent at which AK progresses to SCC have been demonstrated up to 16% (Glogau 2000). Approximately 60% of SCCs have been demonstrated to arise from preexisting AKs or the contiguous skin surface (Sober and Burstein 1995). Therefore, AK can be defined as a potential risk factor for the development of SCC.

Based on histological features, melanoma development has been described by Li and Herlyn as follows: (1) common acquired and congenital nevi with normal melanocytes that have a finite lifespan and no cytogenetic abnormalities; (2) DN that display both cellular and architectural atypia; (3) radial growth of a melanoma; (4) vertical growth phase of the primary melanoma; and (5) metastatic melanoma (Li and Herlyn 2000).

In 10 of 11 case-control studies, DN has emerged as one of the most important risk factors for melanoma (Greene 1997). On average, 34% of patients with melanoma had DN, in comparison with 11% of control subjects. Relative risk ranged from 1.0 to 16.7 for melanoma in the presence of DN. Several studies also reported an increased risk for melanoma with an increase in the number of DN. Cohort studies of patients with familial DN have also provided evidence for presence of DN as a risk factor for the development of new melanomas (Greene 1997). In a retrospective study drawn from 820 patients diagnosed with a first primary cutaneous melanoma, 82% of 50 examined patients with multiple melanomas were clinically diagnosed with DN (Stam-Posthuma et al. 2001). Histological confirmation was demonstrated in 78.0% of these patients and 16 of 37 patients had more than 30 clinically diagnosed DN, eight patients had 11–20 DN, four patients had 21–30 DN and nine patients had one DN. Finally, prospective studies have concluded that patients with DN and no family history also have an increased risk of melanoma (Greene 1997).

Other studies have investigated the idea that melanoma actually arises from DN (Marras et al. 1999). One such report performed cytogenetic analyses of DN in a young patient with a family history of melanoma (Marras et al. 1999). A t(6;15)(q13;q21) translocation found in one of the DN was similar to a translocation, with a breakpoint at 6q13 reported in a benign, non-dysplastic nevi (Richmond et al. 1986) and in a cutaneous metastatic melanoma (Thompson et al. 1995). The repeated occurrence of this rearrangement provides initial support for the hypothesis that melanoma progresses from normal melanocytes to benign nevus, to DN, to early melanoma, to late melanoma, and then to metastatic melanoma.

In a study by investigators at the National Cancer Institute (NCI) and the University of Pennsylvania, almost all members of a family cohort with melanoma also had DN. New melanomas were only diagnosed in family members with DN (Greene et al. 1985a,b). These data suggest that not only are DNs risk factors for melanoma, but they may also be the precursor lesions from which new melanoma evolve.

The use of dysplastic nevi, as a precancerous lesion and an indication of chemoprevention efficacy, has been used in previous research and is proposed in upcoming trials. To date, four chemoprevention trials with topical tretinoin have been performed on individuals

with DN (Stam-Posthuma 1998). In these trials, DNs were targeted as surrogate markers for chemoprevention of melanoma.

### 11.4.4 Molecular Targets for Chemoprevention Identified in UVR Signaling Pathways

Skin carcinogenesis caused by UVR is a multi-step process of initiation, promotion and progression (Fig. 1). The best phases to intervene are the tumor promotion and progression phases, which are slow, rate-limiting stages. The initiation phase occurs rapidly. The targeting of AK and DN are at the promotion phase where several specific genetic alterations can occur. In order to produce specific chemopreventive agents it is necessary to first identify important molecular targets, which are modified in the carcinogenesis process. Bode and colleagues (Bode and Dong 2004) describes three major criteria for "valid" targets for cancer prevention: (1) The target molecule is deregulated in tumor development. The target molecule is affected by a tumor promoter resulting in a cascade of activation or inhibition of signal transduction pathways implicated in carcinogenesis; (2) The outcome of the deregulation of the target molecule results in malignant transformation, cell proliferation, cell cycle arrest and/or apoptosis; (3) The deregulation of the target molecule significantly impacts carcinogenesis in vivo. For both NMSC and melanoma many of these targets can be identified by understanding the UVR signaling pathways and identifying the points where alterations occur due to UVR signaling. This chapter briefly discusses select targets which are discussed in more detail in two extensive reviews of chemoprevention

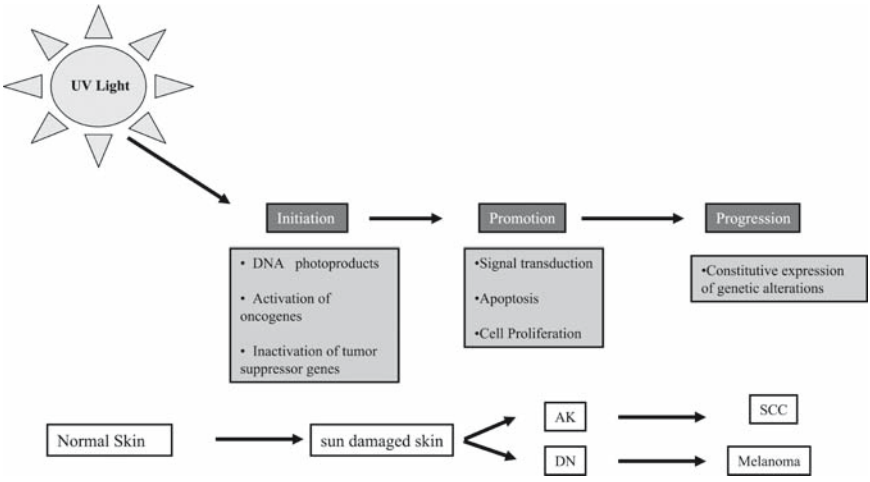


**Fig. 1** Multistep UV-induced carcinogenesis. This multistep process involves sun exposure of normal skin or benign nevi to develop into AK or DN and progress to SCC or melanoma, respectively. The process involves initiation, promotion and progression via the formation of photoproducts, activation of oncogenes or inactivation of tumor suppressor genes to signal transduction, cell proliferation or apoptosis and finally to constitutive activation of genetic alterations

of photocarcinogenesis by targeting UVR signaling (Afaq et al. 2005; Bowden 2004). Bachelor and Bowden have recently reviewed UVA mediated signaling which may be involved in skin tumor promotion and progression and eventually provide additional targets for chemoprevention (Bachelor and Bowden 2004). The identification of these targets has been revealed by in vitro and in vivo model systems in the laboratory. The initiating events in skin cancer appear to involve gene mutations in proto-oncogenes or tumor suppressor genes. In the case of UVR induced skin carcinogenesis, these initiating mutations have been identified in the TP53 tumor suppressor gene as UVR signature mutations (Ziegler et al. 1993). These mutations have been identified in AK and SCC (Nelson et al. 1994). The initiated cell undergoes a clonal expansion during the promotion phase at which point it is most likely that AK and DN in human skin arise. UVR tumor promotion is carried out by signaling molecules that give rise to altered gene expression. For SCC development, the UVR-induced clonal expansion signaling has been demonstrated to lead to the activation of activator protein-1 (AP-1) transcription factor or to cyclooxygenase-2 (COX2) expression (Bowden 2004). Three signaling molecules identified in the UVR signaling cascade (Fig. 2) during the promotion stage of SCC include mitogen-activated protein kinases (MAPKs) (Chen et al. 2001), phosphatidylinositol 3-kinase (PI3 K) (Kabayama et al. 1998), and epidermal growth factor (EGF) receptors (Wan et al. 2001a,b). These three molecules serve as excellent targets for the development of chemoprevention.

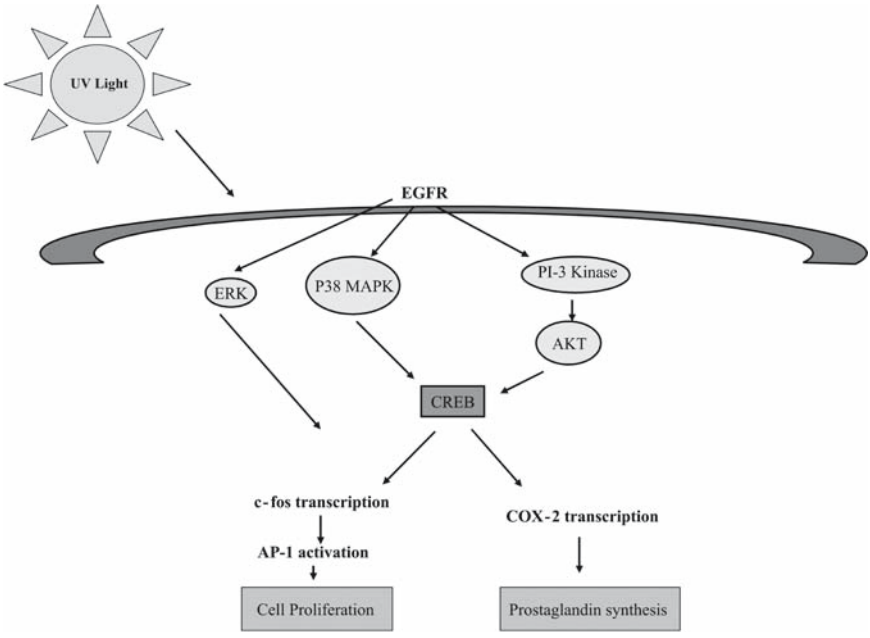


**Fig. 2** UV signaling pathway via the EGFR involves ERK, MAPK and PI-3 kinase activation that leads to the activation of AP-1 and/or COX-2. Theses markers have been identified as potential targets for chemoprevention in NMSC. Current ongoing investigations are exploring a UV signaling cascade including PI-3 kinase, AKT, MAPK and Raf as potential chemoprevention targets for melanoma

AP-1 is upregulated in response to UVR-induced MAPK signaling in human keratinocytes in vitro (Chen and Bowden 2000). The transcription factor, AP-1, mediates the transcription of genes containing a 12-O-tetradecanoylphorbol-13-acetate (TPA) response element (TRE) (Lee et al. 1987). AP-1 is made up of homo- and heterodimers of proteins from the Jun and Fos families (Curran and Franza 1988). These genes are considered early response genes because of their rapid response to environmental changes, such as growth factors, stress, or DNA damage (Angel et al. 1988; Ryseck et al. 1988). Induction of Jun and Fos results from post-translational modification (Stein et al. 1992). In contrast, UVR activation of AP-1-dependent genes, such as the metalloproteinase genes and c-Fos appears to require new protein synthesis of Jun and Fos (Konig et al. 1992).

c-Fos is constitutively expressed in both rodent and human epidermis (Basset-Seguin et al. 1990; Fisher et al. 1991) as demonstrated by immunohistochemical localization, suggesting that c-Fos has a role in growth and cell proliferation (Basset-Seguin et al. 1990). Additionally, UVB (Chen et al. 1998) and UVA irradiation (Silvers and Bowden 2002) has been shown to induce c-Fos expression in human keratinocytes. Similarly, in rat epidermis, single doses of UVB produced a rapid and sustained increase in c-Fos and c-Jun mRNA and protein throughout the epidermis at early time points, but were restricted to the basal layer at later time points. This suggests their possible role in the induction of both apoptosis and cell proliferation (Gillardon et al. 1994).

Investigators have demonstrated the important role of AP-1 in UVR carcinogenesis in human and mouse keratinocytes as well as transgenic mice. UVB (Chen et al. 1998) and UVA (Silvers and Bowden 2002) were shown to activate AP-1 in HaCaT cells (human keratinocyte cell line) with a correlative increase in c-Fos expression. The blocking of AP-1 transactivation in malignant mouse SCC cell lines inhibits the formation of tumors in athymic nude mice (Domann et al. 1994). c-Fos and junD were identified as the main components of the AP-1 complex induced by UVB. The upregulation of AP-1 by UVB has been demonstrated in mouse skin (Barthelman et al. 1998b) and human skin (Fisher and Voorhees 1998). In studies with mouse epidermal JB6 cells, it has been demonstrated that blocking tumor promoter-induced AP-1 activity inhibited neoplastic transformation by an AP-1-inhibiting dominant negative Jun (Dong et al. 1994). UVB irradiation studies have demonstrated an induction of AP-1 through the MAPK signaling cascade in human keratinocytes (Chen and Bowden 1999). A critical role for MAPK signaling (p38 and JNK) in AP-1 transactivation has also been demonstrated by UVA irradiation (Silvers et al. 2003). A mouse model that was used for testing the hypothesis that AP-1 activation has a functional role in the promotion of UVB-induced skin tumors is a TAM67 mouse crossed with a mouse expressing an AP-1 luciferase reporter gene. The TAM67 transgenic mouse contains a dominant-negative c-JUN mutant transgene (TAM67) under the control of the human keratin 14 promoter expressed in the epidermis of SKH-1 hairless mice. These mice show a decrease in UVB light-induced AP-1 activation with a signal UVB exposure. The expression of the TAM67 delayed the appearance of tumors, reduced the number of tumors per mouse and reduced the size of the tumors subsequent to chronic UVB exposure. The data demonstrated that the expression of the TAM67 inhibited UVB-induced AP-1 activation in the epidermis and inhibited UVB-induced skin tumor development. Information gathered from these studies have enabled the formulation of a UVB signaling pathway that leads to AP-1 activation and provides a good molecular target for

the development of new chemoprevention strategies to prevent UVR-induced skin cancers (Bowden 2004).

The MAPKs are part of signaling cascades that involve the regulation of cell proliferation and differentiation in human epidermis (Geilen et al. 1996). Mitogen-activated protein (MAP) kinases are a family of serine/threonine protein kinases. These kinases have been found to be important in cellular response to growth stimuli (Peyssonnaux and Eychene 2001). MAP kinases are activated by translocation to the nucleus, where kinases phosphorylate their targets substrates such as transcription factors (Coso et al. 1995). The MAP kinase family includes c-Jun-$NH_2$ terminal kinases (JNKs/SAPKs), extracellular signal-regulated protein kinases (ERKs) and p38 MAP kinases. JNKs/SAPKs and p38 kinases are activated by stress, including UVR irradiation (Kallunki et al. 1994). Investigators have demonstrated that UVA and UVB irradiation causes activation of ERKs, JNKs and p38 kinases in cell culture (Huang, 1997; Huang et al. 1997a,b,c; Dong et al. 1998).

p38 MAP kinase plays an important role in UVB-induced c-Fos expression in human keratinocytes (Chen and Bowden 1999). Both p38 and ERK were significantly activated by UVB irradiation in human keratinocytes. Treatment of these cells with a p38 inhibitor, SB202190, inhibited UVB-induced p38 activation but did not induce ERK activation. In addition the treatment of the cells with MEK1 inhibitor, PD98059, inhibited UVB-induced ERK activation but not UVB-induced p38 activation (Chen and Bowden 1999). The blocking of p38 almost completely abrogates UVB-induced c-Fos gene transcription and c-FOS protein synthesis. Inhibition of ERK partially abrogates UVB-induced c-fos transcriptional and protein levels. Inhibiting both p38 and ERK completely blocked UVB-induced c-fos expression but also decreased c-Fos basal gene expression. The p38 inhibitor, SB202190, strongly inhibited UVB-induced AP-1 transactivation as well as AP-1 DNA binding (Chen and Bowden 2000). Studies with UVA demonstrated UVA-induced p38 MAP kinase activity also plays an important role is the survival of keratinocytes (Bachelor and Bowden 2004). The inhibition of p38 MAP kinase by SB202190 decreases expression of Bcl-$X_L$ and results increased apoptosis. Consequently, it was shown that UVA-induces p38 MAPK activity and the subsequent increae in Bcl-$X_L$ resulted in a resistance to UVA-induced apoptosis. These data together suggests that the upstream molecules of c-Fos and AP-1 signaling, p38 and ERK are potential targets for chemoprevention in NMSC.

Another target gene in UVB signaling is COX-2. COX-2 is a key enzyme involved in the synthesis of prostaglandins. Prostaglandins have been linked to several important events of the carcinogenesis process. Studies of malignant melanoma progression have demonstrated that no COX-2 expression was observed in dysplastic nevi, primary skin melanoma cells, vertical and radial growth phase cases but COX-2 was strongly detected in the metastatic cancer cells (Goulet et al. 2003). In addition 5 out of 7 melanoma cell lines over-expressed COX-2 compared to normal melanocytes. An increase in COX-2 expression occurs after UVB exposure in both human skin (Buckman et al. 1998) and cultured human keratinocytes (An et al. 2002). There is also an increase expression of COX-2 protein in human SCC biopsies and when compared to normal non-sun exposed control skin. Selective inhibition of COX-2 in hairless mice has resulted in a significant reduction of UVR-induced skin tumors in hairless mice (Pentland et al. 1999). Of particular interest is another study which demonstrated that p38 is

required for UVB-induced COX-2 gene expression in human keratinocytes (Chen et al. 2001). Inhibition of p38 with SB202190 markedly inhibited UVB-induced COX-2 mRNA. There was no effect when the Mek inhibitor PD98059 was used. UVA has also been shown to induce COX-2 in keratinocytes (Bachelor et al. 2002). Since p38 MAPK appears to be an important step in two UV-induced signaling pathways (ending in the transcription factors AP-1 and COX-2), it is an excellent candidate as a target for chemoprevention.

JNK phosphorylates c-Jun (Derijard et al. 1994; Kallunki et al. 1994), a component of the AP-1 transcription factor. There are three JNK genes (JNK-1, -2 and -3) that have been identified in humans. It has been demonstrated that JNK2 knockout ($JNK2^{-/-}$) mice, in a two-stage tumor promotion skin carcinogenesis model with DMBA and TPA, exhibited significant reduction in papilloma burden compared with wild-type controls (Chen et al. 2001). Further studies to look at the UVR signaling pathway for skin carcinogenesis may point toward JNK as another potential target for chemoprevention of skin cancer.

The phosphatidylinositol-3 kinase (PI-3 kinase) pathway regulates cellular proliferation, growth, apoptosis and cytoskeletal rearrangement. PI-3 kinases are heterodimeric lipid kinases composed of regulatory and catalytic domains (Vivanco and Sawyers 2002). PI-3 kinase is an important enzyme associated with a variety of receptors or protein-tyrosine kinases and acts as a direct biochemical link between a novel phosphatidylinositol pathway and a number of receptor proteins, including the receptors for insulin or platelet-derived growth factor (Downes and Carter 1991). This enzyme is a heterodimer of a 110-kDa unit (Auger et al. 1989). It can phosphorylate phosphatidylinositol (Ptdins), Ptdins (4) phosphate [Ptdins (4) P], or Ptdins(4,5) bisphosphate [Ptdins(4,5)P2] to produce Ptdins(3)P, Ptdins(3,4)P2, or Ptdins(3,4,5) trisphosphate [Ptdins(3,4,5)P3], respectively (Whitman et al. 1988; Cohen et al. 1990; Nomura et al. 2001a). Insulin or growth factor stimulation of the associated tyrosine kinase results in phosphorylation of the p85 subunit of PI-3 kinase. This phosphorylation is important for activation of PI-3 kinase (Huang et al. 1997a,b,c). Akt works downstream in the PI-3 kinase pathway to regulate proliferation, apoptosis and growth (Vivanco and Sawyers 2002). Akt, a serine/threonine kinase, is activated by recruitment to the plasma membrane. Clinical evidence of PI-3 kinase activation has been reported in various cancers and the identification of downstream kinases provides a potential target for mediating tumorigenesis (Vivanco and Sawyers 2002). Investigators have shown that UVB irradiation activates Akt in JB6, mouse epidermal cells. This activation was attenuated by inhibitors for MAP kinase/ERK kinase-1 and p38 (Nomura et al. 2001a). It has been reported that PI-3 kinase plays an important role in UVB-induced AP-1 and Akt activation (Huang et al. 1997a,b,c; Nomura et al. 2001a). Inhibition of PI-3 kinase was found to block UVB-induced activation of p90 ribosomal protein S6 kinase (P70S6K), known to be associated with AP-1 in tumor promoter-induced cell transformation (Zhang et al. 2001).

Wan and colleagues demonstrated that solar UVR irradiation of human skin activated epidermal growth factor receptor (EGFR) as well as other downstream signals including MAP kinases, ERK, JNK and p38 (Wan et al. 2001a,b). Their investigations revealed activation of the PI3-kinase/AKT survival pathway via EGFR. They also found that EGF crosstalks with cytokine receptors such as IL-1 receptor leading to the activation of c-Jun kinase in response to UVR irradiation of human keratinocytes. Additional investigators

have shown that UVA-induced EGFR signaling is required for activation of p90RSK/p70S6 K, PI-3 kinase and ERK (Zhang et al. 2001).

Signaling cascades due to UVR stimulation that leads to skin carcinogenesis of melanoma are not defined as extensively as for NMSC. Investigators have outline UVR signaling pathways for melanogenesis (Tada et al. 2002). However, there is a thus far only a few identified molecules that could potentially serve as molecular targets (Raf and MAPK) for chemoprevention of melanoma. The Raf kinases were the first Ras effectors identified and have been the most extensively studied (Hunter 1997). Ras associates with and activates Raf-1, which in turn phosphorylates and activates MEK kinase, which in turn phosphorylates the MAP kinases, ERK1 and ERK2 (Liaw et al. 1993; Samuels et al. 1993; Warne et al. 1993; Ghosh et al. 1994). Activated MAP kinases translocate to the nucleus where they can modulate gene expression (Hill and Treisman 1995; Marshall 1995). Raf-1 has also been shown to interact with PKC, a key regulatory protein associated with a second signal transduction pathway (Kolch Heidecker et al. 1993). Two well-established biological events that are associated with activation of the Raf/MEK/ERK pathway are cell proliferation and cell cycle progression. Halaban and colleagues have observed that several of the mitogenic factors for melanocytes, bFGF, MCGF, and HGF/SF, stimulate ERK1/ERK2 phosphorylation (Halaban et al.1992a,b; Funasaka et al. 1992). Others have demonstrated that Raf plays an important role in progression of melanoma (Pollock et al. 2003). The data from these studies identified a particular mutation that was found in 68% of metastatic melanoma, 80% of primary melanoma and 82% of a diverse set of nevi. These findings implicate Raf as a potential target for chemoprevention of melanoma, since Raf mutations are evident at the early stage of primary melanoma and nevi. The identification of MAPK as an early event in melanoma progression (Cohen et al. 2002) provides another potential target for the chemoprevention of melanoma.

A recent review by Zhang and Bowden (Zhang and Bowden 2007) describes the targeting of Bcl-$X_L$ for both the prevention and therapy of skin cancer. Bcl-$X_L$ is localized on the mitochondrial outer membrane and plays a critical role in the homeostasis of both the intrinsic and extrinsic apoptotic pathways. Several studies have shown an antiapototic role of Bcl-$X_L$ in skin. In immortalized keratinocytes,Bcl-$X_L$ has been shown to be protective against UVA-induced apoptosis (Bachelor and Bowden 2004). Zhang and Bowden describe the plausibility of targeting Bcl-$X_L$ for NMSC and melanoma (Zhang and Bowden 2007). Melanoma cell lines have shown higher expression of Bcl-$X_L$ than melanocytes (Bush and Li 2003) and both primary and metastatic melanoma have demonstrated increased expression (Zhang and Rosdahl 2006). Bcl-$X_L$ has been shown to render primary and metastatic melanoma cells resistant to UVB irradiation. In a chemically-induced skin carcinogensis mouse model, expression of Bcl-$X_L$ in a transgenic mouse resulted in a twofold increase in the number of papillomas formed compared to the wild-type mouse (Pena et al. 1998). In addition more than half the transgenic mice developed SCC within 7 months of treatment while none of the wild-type mice had SCC in the same time. The critical role in several stages of skin carcinogenesis, including initiation and promotion, make Bcl-$X_L$ a very plausible target for prevention of skin cancer through the development of chemopreventive agents.

### 11.4.5 Animal Models for Studying Chemoprevention Agents

In order to understand the mechanism of carcinogenesis and investigate efficacy of chemoprevention agents prior to clinical application, animal models that closely resemble human disease must be developed. The SKH-1 hairless mouse is a model for the studies of skin cancer pathogenesis and the evaluation of chemoprevention of UVB-induced skin cancer (Bowden 2004). The most obvious advantage of these mice is that they are hairless and therefore do not require any removal of hair that may actually protect the skin from UVR light. With increasing dose level, three times a week for 25 weeks nearly 100% of the mice develop at least one skin tumor with an average of seven to nine tumors per mouse. Most of these tumors are SCC, which arise from benign papillomas. UVB irradiation is used as a complete carcinogen in these mice. Another protocol used with these mice is UVB exposure twice a week for 20 weeks. This results in epidermal hyperplasia; no immediate tumors occur but a high risk of developing skin tumors during the next several months in the absence of any further UVR. This latter model system resembles humans who are heavily exposed to UVR early in life with reduced exposure later in life. Chemoprevention agents can be tested in these models.

A mouse strain with abnormalities in the hedgehog signaling pathway develops neoplasms which closely resembles human BCC. These mice contain a heterozygous allele in the PTCH gene (Ptc+/−). Chemoprevention studies with green and black tea have been studied in this mouse model (Herbert et al. 2001).

Multiple animal models of melanoma have been reported however difficulties with these models for studies of chemoprevention are that tumors develop at a low incidence rate and the latency period is often very long. There are two prominent models for melanoma, which are useful for the studies of chemoprevention agents. Powell et al. (1999) report the development of a transgenic mouse for which when chemically induced develops melanoma. The mouse line expresses a mutated human Ha-ras (TPras) gene driven by a mouse tyrosinase promoter. This transgene is therefore expressed in pigment producing cells of the mice. The protocol for inducing melanoma in these mice is topical application of 50 μg 7,12-dimethylbenz-[a]anthracene (DMBA) once a week for five weeks. Development of melanoma occurs around 15 weeks. Tumors only occur in the mice expressing the transgene and no tumors develop in the negative littermates. Tumors develop in more than 80% of the treated mice. No spontaneous cutaneous melanoma or other skin cancers develop in these mice. Metastatic lesions have been observed in the skin, lungs and lymph nodes of the DMBA-treated transgenic mice (Powell et al. 1999). Melanomas isolated from TPras transgenic mice display alterations and/or losses of p16 (Gause et al. 1997) much like human melanoma. Early experiments with these TPras mice did not result in UVR induce melanoma perhaps because of the highly pigmented skin of the adult TPras mouse. Further investigations using this model have found ways to indeed produce UVR-induced melanoma in this model system. The first was a single neonatal exposure (2–3 day old mice) of UVR light which resulted in a penetrance of 57% by 12 months (Hacker et al. 2005). Another development of this model has been to cross it with an activated Cdk4 mouse. This resulted in spontaneous melanomas with a increase of penetrance of 83% when treated with UVR (Hacker et al. 2006). Another model

is a transgenic model, which utilizes a metallothionein-gene promoter driving a hepatocyte growth factor/scatter factor (c-Met receptor tyrosine kinase ligand) gene based on the albino FVB background (Noonan et al. 2001). Development of melanoma occurs in this model system after a single acute exposure of an erythemal dose of UVR irradiation. Development of invasive melanoma occurs in 80% of the animals. These melanomas closely resemble human melanoma in terms of the development between the dermis and epidermis.

One concern with UVR studies is the ability of the experimental UVR exposure to imitate the true solar spectral output. Many studies with UVR use light sources which produce primarily UVB output with minimal UVA output. One recent debate has been brought forward in a study by Ibuki and colleagues (Ibuki et al. 2007) which produced data that suggested that UVA produces a protective role against UVB by inhibiting UVB induced apoptosis. However, a published commentary (Runger 2007) to this publication noted that since the UVA radiation inhibits UVB-induced apoptosis that this may only increase the mutation burden that would normally be eliminated by UVB induced apoptosis and therefore increase skin cancer formation. With these questions still left to be answered, it is best to use UVR sources which combine both UVA and UVB spectral output which best mimics the solar output for any studies of chemoprevention agent in animal models.

### 11.4.6 Endpoints for Evaluating Efficacy of Chemoprevention Agents

Because the process of carcinogenesis can take many years, assessment of clinical chemoprevention trials using cancer incidence as an endpoint requires a long follow-up period and large sample sizes. In addition to evaluating the modulation of targets for a specific chemopreventive agent such as those involved in the UV signaling pathway discussed above (p38 MAPK, PI-3 kinase etc), biomarkers are useful for evaluating the efficacy of a chemopreventive agent. The rationale for the use of intermediate biomarkers is to circumvent these issues in chemoprevention trials (Einspahr et al. 1997), since biomarkers occur at steps preceding the occurrence of malignancy. As discussed by Lippman and colleagues (Lippman et al. 1990), biomarkers of intermediate endpoints can be defined as measurable markers of cellular or molecular events associated with specific stages of the multi-step progression of carcinogenesis. Thus, the risk of carcinogenic transformation, whether in the skin or other sites, can be correlated with the quantitative degree and pattern of biomarker expression. Criteria for identifying and evaluating the potential efficacy of biomarkers are as follows:

- Variability of expression between phases of the carcinogenesis process (i.e., normal, premalignant, malignant)
- Ability for early detection in the carcinogenesis pathway
- Association with risk of developing cancer or recurrence of the precancer
- Potential for modification by a chemopreventive agent
- Presence in tissues that are easily accessible for multiple biopsies
- Capability to develop adequate assay quality control procedures

Markers of cellular proliferation can be used as intermediate biomarkers to evaluate the efficacy of chemoprevention agents in clinical trials and animal model systems. Enhanced cellular proliferation has been closely associated with the process of tumorigenesis in numerous tissues including skin (Einspahr et al. 1996). Proliferating cellular nuclear antigen (PCNA) functions as an auxiliary protein to DNA polymerase d and e in DNA replication and repair (Hall et al. 1990). Expression of PCNA increases late in G1, is maximally expressed in S, and decreases in the G2/M phases of the cell cycle. Therefore, PCNA can be used to evaluate cell proliferation and possibly chemoprevention efficacy. Studies have indeed found a significant difference in PCNA expression in AK compared to sun-damage skin (Einspahr et al. 1996, 2006) but not sun-damaged forearms compared to forearms from subjects with AK (Einspahr et al. 2006). PCNA was not useful in detecting an effect of the chemoprevention agent difloromethylornithine (DFMO) (Einspahr et al. 2002). These investigators suggest that PCNA may be useful in combination with the number of apoptotic cells as an endpoint for clinical trials with chemoprevention agents. Another extensively used marker for proliferation is Ki67 which is present is all active phases of the cell cycle (G1, S, G2 and mitosis) but is absent from resting cells. MIB-1 is a commonly used monoclonal antibody that detects the Ki-67 antigen. Bordar and colleagues (Bordbar et al. 2007) evaluated the MIB-1 antibody in its usefulness in differentiating benign, premalignant and malignant skin lesions.

Apoptosis also serves as biomarker for the efficacy of chemoprevention agents in clinical trials and animal model systems. Apoptosis is a unique mode of cell death, characterized by ultrastructural changes distinct from necrosis (Kerr et al. 1972). In the developing animal, programmed cell death removes cells during remodeling of a number of organs (Haake and Polakowska 1993). Apoptosis is also involved in tissue regression following hormone stimulation or deprivation in hormone-sensitive tissues, such as the prostate, and functions in development of the immune system (Haake and Polakowska 1993). In continually renewing tissues such as the epidermis, homeostasis is maintained through a balance between cellular proliferation and cell death. Apoptosis may also play an important role in regression of neoplasms (Haake and Polakowska 1993). Alterations in either cell proliferation or cell death can lead to loss of growth control, thereby playing major roles in the process of tumorigenesis. Apoptosis is characterized by cell shrinkage, plasma membrane blebbing, nuclear fragmentation and chromatin condensation. Apoptotic cells are rapidly phagocytosed by neighboring cells in order to prevent the release of cell contents. In contrast to necrosis, apoptosis is an organized and controlled process of cell death (Kerr et al. 1972). Measurements for apoptosis may include morphology, in situ TUNEL and caspase-3 detection.

Investigators have shown that p53 mutations increase through the progression of normal skin, sun damaged skin, AK and SCC. While the frequency of p53 mutations was 14% in normal skin, this percentage rose to 38.5% in sun-damaged skin, 63% in AK and 54% in SCC. Proliferation was also increased through this same progression. SCC samples demonstrated an increased presence of BAX compared to AK (Einspahr et al. 1999). An additional study confirmed the use of p53 expression as a valid biomarker in the progression of sun damage skin to AK to SCC (Einspahr et al. 2006). This latter study demonstrated that p53 expression as well as expression of selected polyamines is effective in differentiating early stages of skin cancer progression and was not affected by sunscreen use. These data

supports the use of p53 as a biomarker for disease progression when evaluating the efficacy of chemoprevention agents. A recently presented study demonstrated that vascular endothelial growth factor (VEGF) may serve as a biomarker for detection and chemopreventive modulation for melanoma studies. Investigators found that there was a higher VEGF expression in dysplastic nevi compared to benign nevi (Thomas et al. 2006). Biomarkers that measure micronutrient and biochemical levels in tissue and blood may also be useful biomarkers in studies that evaluate chemopreventive agents' ability to slow or inhibit progression from a benign to premalignant to malignant stage.

Karyometric evaluation of the epidermis has been used as a developmental secondary endpoint in clinical studies (Bozzo et al. 2001). Ranger-Moore and colleagues have published a complete review of karyometric measures in intraepithelial lesions discusses the usefulness of karyometry as an integrating biomarker for evaluating progression and effectiveness of chemopreventive agents (Ranger-Moore et al. 2005; Bartels et al. 2006). The advantage to this type of biomarker is that it can detect activity of a chemopreventive agent even when the mechanism for a given progression pathway is unknown or when multiple pathways exist. Nuclear chromatin patterns can be used diagnostically to assess changes in the development of cells, particularly the development into a cancerous cell, which could then be correlated with the prognosis of individual patients. Image analysis of nuclear chromatin patterns provides a quantitative approach (Weyn et al. 2000). With image analysis, karyometric features are described by the arrangement of a combination of pixels. These features are then combined by means of multivariate analysis of criteria used for prognosis. Digital microscopic studies of epithelia from ectocervix (Wied et al. 1980), lung mucosa (MacAulay et al. 1995), colonic mucosa (Bibbo et al. 1990), glandular epithelium of the thyroid (Bibbo et al. 1986), breast (Susnik et al. 1995), bladder (Sherman and Koss 1983), and the prostate (Irinopoulou et al. 1993) have detected very subtle, possibly pre-neoplastic changes in the organization of nuclear chromatin in biopsies from individuals with premalignant and malignant lesions of these organ sites. When these same tissue sections were examined with standard histopathological techniques, no abnormalities were detected. Thus, digital microscopy can provide highly sensitive detection of early change and may provide novel diagnostic clues. Digital imagery can reliably detect very early subtle changes in the organization of nuclear chromatin in epithelial cells that appear to be entirely normal during histopathologic examination. This technology, which uses high resolution imagery of cell nuclei to assign values to karyometric features, may enable the quantitative assessment of progressive change from normal appearing to severely sun damaged skin to AK to SCC as well as from DN to melanoma. Nuclear karyometric measurements have been performed on both benign and malignant melanocytic lesions (Bjornhagen et al. 1994; Stolz et al. 1994). Using imprint specimens, Stolz and colleagues (Stolz et al. 1994) found five features (mean value and standard deviation of nuclear area and the 80th, 90th and 95th percentile of the DNA distribution) to be significantly different between benign melanocytic lesions and melanoma. A second report (Stolz et al. 1994) found significant differences between benign melanocytic tumors and malignant melanoma for the following features: mean nuclear area; coefficient of variation (cv) of nuclear area; cv of nuclear shape; nuclear contour index; mean and cv of nucleolar area; and DNA distribution rates. Investigators have conducted feasibility studies for the karyometric assessment of skin shave biopsies of AKs and for the assessment of the effects of chemopreventive intervention, using

quantitative characterization by digital microscopy (Bozzo et al. 1998). Sections of shave skin biopsies were digitized and a minimum of 100 nuclei from each was recorded per case. After image segmentation, feature extraction software produced 93 karyometric features per nucleus that were stored for analysis. Discriminant functions were derived according to differences between normal nuclei and those with sun damage. Profiles commonly found in malignant cells were seen in the AK lesions. Using these features, a grading score was developed based on a plot of degree of solar damage versus the mean discriminant function. While upper inner arm (minimally sun exposed) skin biopsies demonstrated as few as 3% of nuclei affected by sun damage, the AK lesions included approximately 50% affected nuclei. Discriminant functions derived from values obtained from samples ranging from normal to sun damaged to premalignant (AK or DN) to malignant (SCC or melanoma) phenotypes establish a progression curve that can be used to determine the efficacy of applied chemopreventive agents (Bozzo et al. 2001) (Fig. 3). They have also applied this novel technology to demonstrate the efficacy of two chemoprevention agents, α-DFMO and Vitamin A, in patients with moderately severe sun damaged skin (Bozzo et al. 2001; Alberts et al. 2004).

Optical coherence tomography (OCT) is a potentially new technique for identifying and characterizing AKs and monitoring their response to chemoprevention agents (Barton et al. 2003). Based on Michelson interferometry, this technique was first introduced for investigations of the human eye (Fercher et al. 1988). This non-invasive technique uses coherent light operating in the near infrared region of 1,300 nm to produce two-dimensional images of the skin (Welzel 2001). The resulting photons have a typical penetrating depth of 1.0–1.5 mm allowing for multiple layers and structures to be distinguished. The resolution

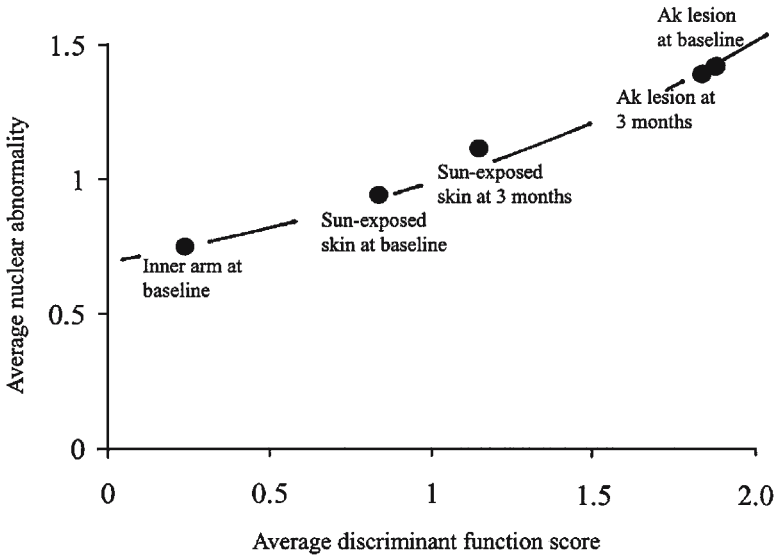


**Fig. 3** Average nuclear abnormality versus average discriminant function scores for the 10% worst nuclei from the upper inner arm at baseline, sun-exposed skin at baseline and 3 months, and AK lesions at baseline and 3 months (Ranger-Moore 2002)

afforded by this technique makes it possible to distinguish features such as stratum cornea, epidermal layer, hair follicles, sebaceous glands and blood vessels. In addition, it is possible to evaluate the efficacy of topical application of ointments and similar treatments, as these compounds tend to increase the detection/penetrating depth of the coherent light (Welzel 2001). In OCT, images of epithelial skin tumors and cell aggregation from the epidermis are visible. In some cases, lateral borders of the tumor adjacent to healthy skin are detectable and BCC can be distinguished from fibrous stroma. It is also possible to diagnose various inflammatory skin diseases such as psoriasis and eczema. The OCT measurement is an unobtrusive and safe technique with no side effects for the patient.

In a pilot study on 20 subjects to investigate the OCT appearance of upper inner arm, sun-damaged skin and mild AKs (Barton et al. 2003) and to determine if features or quantitative measures in OCT images could be used to reliably differentiate between these categories, OCT images of upper inner arm showed skin layers and features (stratum corneum, epidermis, dermis, blood vessels) seen in previous studies; additionally in this subject base the subcutaneous fat layer was usually seen. Sun damaged skin was characterized by increased signal in the epidermis and rapid attenuation of light. AKs were diverse in appearance but frequently characterized by high surface reflection, the presence of a low-signal band in the stratum corneum, and heterogeneous appearance in the epidermis/dermis. Significant differences were found between skin categories using measures of stratum corneum and epidermal/dermal depths and intensities. The presence of a dark band in the stratum corneum was 79% sensitive and 100 specific specific for AK. This study suggests that OCT may be a useful non-invasive technique for monitoring AK during the clinical studies to evaluate the efficacy of chemoprevention agents.

### 11.4.7 Potential Chemoprevention Agents for Skin Cancer

The previous chapter (Chap. 10) describes a novel way to deliver chemoprevention compounds to the skin by the development of topical prodrug formulations. Therefore, this chapter focuses primarily on agents that have not yet been discussed in the previous chapter nor the technology used for prodrug formulation of skin chemoprevention agents. However, many of these compounds could also be considered for prodrug formulation. Figure 4 depicts potential UVR induced targets and chemoprevention agents which may act on these targets for the prevention of skin cancer progression. The investigators at the Arizona Cancer Center use a decision tree which results in leads for chemoprevention agents that will potentially result in a clinical trial (Einspahr et al. 2003). The agents are selected based on epidemiological literature and activity in in vitro and in vivo models of UV skin carcinogenesis. Agents with novel mechanisms of action that are active against identified molecular targets are tested for their ability to modify the target and inhibit tumorigenesis in the animal models. Subsequent to toxicological evaluation and proper formulation, promising agents then progress to human phase I and then to phase II trials in subjects with AKs, DNs or sun-damaged skin. Intermediate endpoints are evaluated to identify efficacy of the select agent. The following discussion provides an overview of chemoprevention agents for skin cancer which have been or are currently in clinical

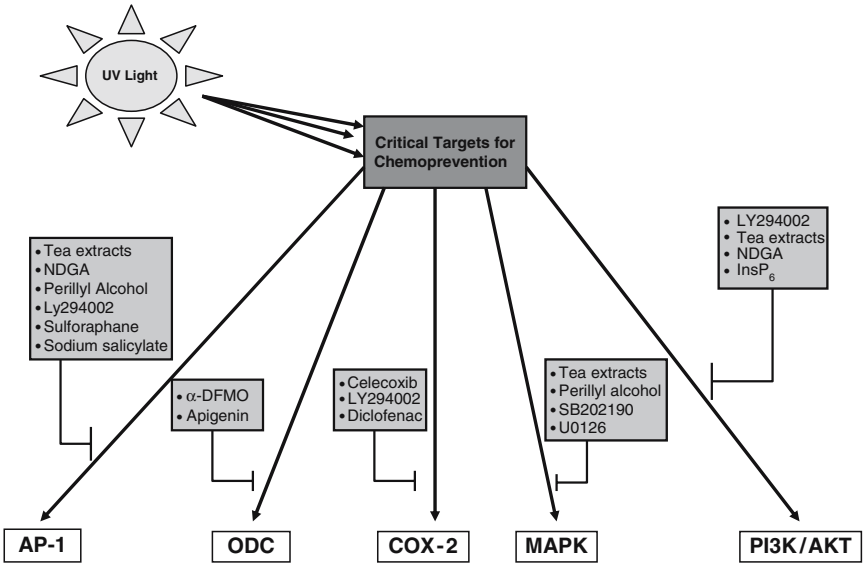


**Fig. 4** UVR induced targets can be modulated by specific potential chemoprevention agents

trials as well as future agents which may result in clinical trials due to their activity toward modulation of molecular targets previously discussed.

Several potential chemopreventive agents have been taken through Phase III clinical trials in people at high risk for NMSC (Stratton 2001; Bowden 2004). The agents include beta-carotene (Greenberg et al. 1990), selenium (Clark et al. 1996), retinol (Moon et al. 1997) and 13-*cis*-retinoic acid (Tangrea et al. 1992). Of these trials, the only one with positive results involved oral administration of 25,000 U per day of retinol in 2,297 subjects with moderate to severe AK (Moon et al. 1997). This trial resulted in a reduction in SCC but not in BCC. The hazard ratio for first new SCC was 0.74 when comparing subjects from the retinol supplemented group to placebo. Vitamin A (retinol) has been demonstrated to be necessary for cell growth and differentiation of human epithelial tissues, reproduction and visual function (Gudas 1994). Retinoids have been shown to be involved in cell growth, cell differentiation and carcinogenesis, all mediated in part by nuclear retinoic acid receptors and retinoid X receptors (Mangelsdorf et al. 1994; Xu et al. 1994).

There have been several smaller phase II trials in subjects at risk for NMSC that have resulted in positive outcomes. Recently, a phase IIa/IIb safety, dose-finding and efficacy study of orally administered Vitamin A in participants with sun damaged skin resulted in a positive outcome (Alberts et al. 2004). The results were evaluated using karyometric analysis (described previously). One hundred twenty randomized participants were given daily oral placebo, 25, 50 or 75 K units of Vitamin A (retinyl palmitate) for 12 months. The primary endpoints included quantitative, karyometric image analysis and assessment of retinoid receptors in sun-damaged skin. This analysis suggests that orally administered

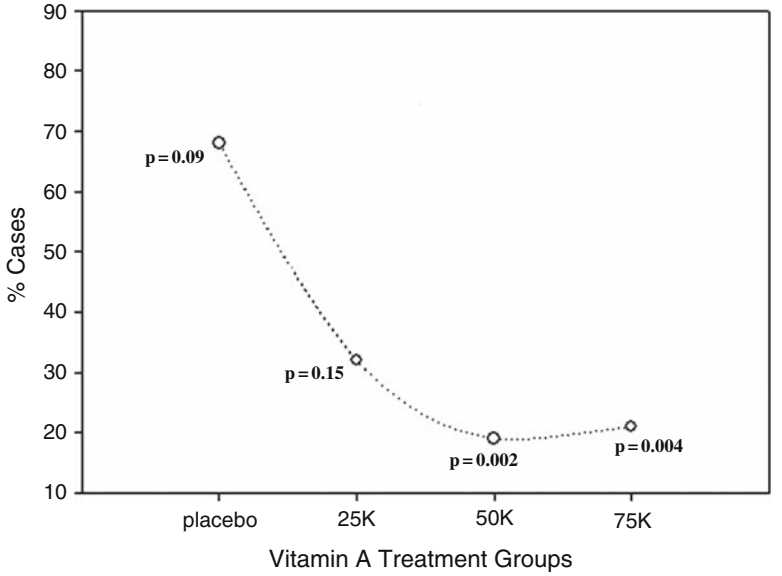


**Fig. 5** Dose response to vitamin A treatment as demonstrated by percent of cases with increased actinic damage decreases based on karyometric analysis, adapted from Bartels et al. (Bartels et al. 2002)

Vitamin A is effective as a skin cancer chemopreventive agent by reducing levels of actinic nuclear damage as measured by average nuclear abnormality levels and discriminant function scores derived from appropriate karyometric features (Fig. 5). The dose effects of Vitamin A correlated with increases in retinoid receptors, RAR-α, RAR-β and RXR-α, at the 50,000 IU per day vitamin A dose.

Another clinical trial performed by Alberts and colleagues demonstrated that topical 2-(Difluoromethyl)-DL-ornithine (α-DFMO) can reduce spermidine concentrations and the number of AK lesions in patients at high risk of skin cancer (Alberts et al. 2000). Forty-eight participants with moderately severe AKs on their forearms were assigned randomly to topical α-DFMO treatment. A reduction of 23.5% in the number of AK lesions was seen from baseline to the 6-month follow up. Spermidine concentration was reduced by 26% in skin biopsies from α-DFMO-treated arms. No systemic toxicities were detected; however, 7 of the 48 (14.6%) participants experienced severe (4.2%) or moderate (10.4%) inflammatory reaction on their α-DFMO-treated arms. In skin biopsies from this study, investigators were able to demonstrate a significant reduction of 22% in p53-positive cells (Einspahr et al. 2002). However, there were no significant changes in proliferation cell nuclear antigen (PCNA) index, apoptotic indices or p53 mutation frequencies. With karyometric analysis, α-DFMO treatment markedly decreased the discriminant function score indicating effectiveness in reducing nuclear abnormalities. α-DFMO is an irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis, and may exert its chemoprevention effects by inhibiting growth and/or inducing apoptosis. α-DFMO inhibits polyamine biosynthesis by covalently binding to ODC,

thus inhibiting proliferation and inducing apoptosis. Leads for the use of α-DFMO came from previous studies where α-DFMO had been demonstrated as an antitumor agent in several animal models for carcinogenesis including a report that oral α-DFMO inhibited cutaneous carcinogenesis and immunosuppression in a mouse model (Gensler 1991). In Xpa knockout mice, α-DFMO, given in drinking water, reduced UVR-induced skin tumors in mice (Takigawa et al. 1990). Tumor-suppressive activity was demonstrated for α-DFMO in melanoma (in vitro and in metastatic melanoma in a clinical trial) (Bregman and Meyskens, 1986; Meyskens et al. 1986).

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) often used alone for degenerative arthritis management or with opioids in the treatment of pain associated with cancer. Investigations have shown that diclofenac has activity in the treatment of AK, thereby potentially preventing progression to SCC. An early study of 29 subjects assessed the efficacy and safety of topical diclofenac (Solaraze). Three percent diclofenac in 2.5% hyaluronic acid gel was generally well tolerated with the exception of seven (24%) patients who experienced irritant-type contact dermatitis (Rivers and McLean 1997). Additional clinical trials have further explored the potential therapeutic effect of this gel formulation. One randomized double-blind controlled trial of 130 patients, which did not include a follow up period, did not find a significant difference between the use of diclofenac/hyaluronan and placebo in the eradication of AKs (McEwan and Smith 1997). However, two other randomized, double-blind, placebo-controlled studies found treatment with 3.0% diclofenac to be effective in the treatment of AK. A study of 195 patients with at least five AKs investigated the duration of treatment for 30 or 60 days with two daily applications of the gel (Rivers et al. 2002). While no significant difference was seen after 30 days, the 60-day treatment group showed a statistically significant difference in the number of patients (33–10%) with complete resolution of all target lesions in treated areas when compared to placebo group. The 60-day treatment group was also significantly different than placebo in the number of patients with resolution of target and new lesions in treated area, visible but no longer palpable lesions and investigator and patient global improvement indices. Another study of 96 subjects also demonstrated a statistically significant difference in a 60-day treatment regimen when comparing the above criteria (Wolf et al. 2001). In both these studies, the gel was well tolerated with only a few subjects reporting skin reactions. The mechanism of action of the 3.0% diclofenac, with regard to tumor resolution, is unknown. As an NSAID, diclofenac inhibits cyclooxygenase enzymes (COX-1 and COX-2). A case control study in women found an inverse association of NSAIDS intake and malignant melanoma (Harris et al. 2001b). One group demonstrated that 26–28 primary melanoma cell lines expressed COX-2 (Denkert et al. 2001).

The COX-2 inhibitor, celecoxib, has been shown to suppress the formation of UV-induced skin cancers when given orally to mice (Pentland et al. 1999; Fischer et al. 1999). Topical application of celecoxib reduced cutaneous inflammation (Wilgus et al. 2000). Reduction of the inflammatory response could prove protective against long term UV exposure and the development of skin cancer and the conversion of benign AK to cancerous ones. An UVR-induced tumor mouse model demonstrated a lengthening in tumor latency period and reduced tumor multiplicity when mice were treated with a COX-2 inhibitor, celecoxib (Orengo et al. 2002). An increase in arachidonic acid metabolism in keratinocytes due to exposure to UVB (Buckman et al. 1998) may be a target for this type

of chemoprevention. A study of a topical combination of celecoxib and 5-FU, a common treatment for AK, found that the combination was 70% more effective in reducing the number of UVB induced skin tumors than with 5-FU alone (Wilgus et al. 2004).These studies indicate a genuine possibility of celecoxib being a chemopreventive agent for skin cancer.

An agent developed at the University of Arizona, Melanotan-1 (MT-1) could potentially be used as a chemopreventive agent against melanoma and NMSC. This agent is a super-potent melanotropic peptide that results in darkening of the skin (Fig. 6). In clinical studies with MT-1 there was no improvement of tanning in doses greater than 0.16 mg $kg^{-1}$ per day for 10 days by subcutaneous (SC) injection. No moderate toxicities occurred at this dose as in the higher doses (Levine et al. 1999); a single exposure of three minimal erythema doses (MED) of UVB did not enhance eumelanin content of the skin either before or after MT-1 administration (Dorr et al. 2000). While treatment showed darkening of the skin on days 14 and 21, there was no significant difference from baseline to four weeks after dosing. Investigators found that the most effective delivery was by SC administration, which resulted in an increase in eumelanin and measured tanning by reflectance in the forearm and forehead. For the purpose of prolonging skin darkening by the use of MT-1, investigators formulated a controlled release MT-1 implant formulation based on a PLGA polymer. In studies with an in vitro frog skin bioassay, they found that the implants (1 mg of peptide) showed the melanophores migrated to the dendritic processes of the pigment cells, which resulted

**Fig. 6** Early clinical trials (Levine 1991) demonstrated skin tanning. *Left*: pretreatment state. *Right*: tanning of the face and neck after start of therapy

in skin darkening. In vivo studies using pigmented haired and hairless guinea pigs with the MT-1 implants (4 mg peptide), increase of skin darkening was observed for up to 3 months and eumelanin content demonstrated a 2.5-fold increase 1 month and persisted for 3 months. This prolonged increase in pigment, specifically eumelanin, can be favorable to prevent photodamage induced by UVR radiation.

Three phase I clinical trials performed at the Arizona Health Sciences center in Tucson, demonstrated that MT-1 can be safely combined with UV-B light or sunlight and appears to act synergistically in the tanning response to light (Dorr et al. 2004 #596). In these studies enhanced tanning was achieved in the groups receiving MT-1 as well as 47% fewer sunburn cells at the irradiated site. Subcutaneous doses of 0.16 mg $kg^{-1}$ per day for 10 days provided an increase darkening and a maintained tanning for at least 3 weeks longer than those exposed to sunlight-exposure alone. The sun exposure time for equivalent tanning in the sunlight only controls required 50% more time for an equivalent tanning. Currently, MT-1 is under development in Australia, licensed by Clinuvel Pharmaceuticals (formerly Epitan, Ltd.) under the proprietary name, CUV1647. Two objectives for the Australian studies have been: (1) to determine the efficacy of MT-1 in at risk skin damage prone populations; and (2) the development of an improved formulation/schedule for delivery, namely a slow release depot formulation designed to release drug from a single subcutaneous injection over several months (Hadley and Dorr 2006). Phase I/II trials in Australia found that the 0.16 mg $kg^{-1}$ per day injection dose caused increased eumelanin deposition in the skin, similar to results reported in the Arizona studies (Fitzgerald et al. 2006). No dose limiting side effects were noted. Of additional interest, patients with variant MC1R genotypes were evaluated for their response to MT-1 to evaluate if it is useful for individuals most in need of photoprotection (Fitzgerald et al. 2006). These variants such as Arg151Cys, Arg160Trp and Asp294His are associated with fair skin color and red hair (Box et al. 1997; John and Ramsay 2002; Valverde et al. 1996). Individuals with one or more of these variants have been shown to be less able to tan naturally with UV light (Healy et al. 2000) and the variants have been associated with an increased risk of skin cancer (Bastiaens et al. 2001; Kennedy et al. 2001; Palmer et al. 2000). The study on the effect of MT-1 on humans with MC1R variant alleles demonstrated that the agent effectively increased the melanin content in the skin of individuals with variant alleles and therefore those most in need of photoprotection (Fitzgerald et al. 2006). A human depot formulation of MT1 (20 mg) successfully produced pigmentation in a pilot phase I study (Hadley and Dorr 2006). Following phamacokinetic studies in healthy volunteers the "controlled release" implants contains 16 mg of MT-1. The increased pigmentation of the skin appears approximately 4–5 days after implantation and may last for several months (www.clinuvel.com). In 2007, this agent began phase III clinical trials for Polymorphic Light Eruption (sun poisoning) and Erythropoletic Protoporphyria (absolute sun intolerance). In November of 2007, Clinuvel Pharmaceuticals initiated a phase II trial in Australia and Europe for CUV1647 as a preventive for sun damage and AK in fair skinned, immune-compromised organ transplant recipients (FDA News 2007). The trial will evaluate the ability of the agent to reduce the number of AK on the head, back of hand and forearms during a 24 month test period. A secondary endpoint will determine the effect of this agent on the number of SCC on the head, back of hand and forearms during the 24 month test period.

Dellavalle and colleagues (Dellavalle et al. 2003) reviewed the role of statins or fibrates in melanoma chemoprevention. Results from two large clinical trials demonstrated a decrease in melanoma incidence in subjects given lipid-lowering medications for coronary artery disease. In another study 27 melanomas were newly diagnosed in 3,301 placebo-treated patients, whereas only 14 melanomas were diagnosed in 3,304 lovastatin-treated patients (Buchwald 1992). The incidence of all other cancers was not statistically different. Another study with gemfibrozil, a hypolipidemic medication, nine melanomas were diagnosed in 1,267 patients treated with placebo and only one melanoma was diagnosed in a 5-year period in 1,264 gemfibrozil-treated patients (Rubins et al. 1999). Again, all other cancers were not significantly different. Statins are known to inhibit the isoprenoid protein modification and therefore may be inhibiting ras farnesylation and cause a downregulation of ras oncogenic effect in melanoma.

Other phase II trials have focused on the potential chemoprevention activity of topical green tea extracts (e.g. polyphenon E) in patients with AK on their arm (Stratton 2001). Animal studies have demonstrated a chemopreventive effect of epigallocatechin gallate (EGCG). Investigators have reported a reduction in tumor incidence with topical application of EGCG in UVB-irradiated mice. Mice were irradiated at a total dose of $2.1 \times 10^6$ J $m^{-2}$. Skin cancer developed in 96% of control mice and 62% of mice given 10 mg of EGCG and 39% of mice given 50 mg of EGCG. EGCG did not affect immunosuppression and oral administration did not decrease UVR-induced skin tumor incidence. In the investigation of a mechanism of action for EGCG, it was demonstrated that EGCG can inhibit UVB-induced AP-1 activity in a dose range of 5.45 nM to 54.5 µM in human keratinocytes when applied before, after or both before and after UVB irradiation (Barthelman et al.1998a). Inhibition of AP-1 by topical EGCG application was also evident in a transgenic mouse model. EGCG inhibited UVB-induced steady state message and transcriptional activation of the c-fos gene as well as the accumulation of the c-Fos protein. Upstream of c-Fos, EGCG significantly inhibited activation of p38 MAPK yet did not affect JNK or ERK activation. AP-1 inhibition potentially, through the reduction of c-Fos by EGCG, may be the mechanism by which EGCG inhibits UVB-induced tumor formation in mice (Chen et al. 1999). Theaflavins from black tea demonstrated a stronger inhibition of AP-1 than EGCG and inhibition of the activation of ERK and JNK was also significant with theaflavin treatment (Nomura et al. 2000). In addition, Nomura and colleagues demonstrated in mouse epidermal cells that EGCG and theaflavins inhibited the activation of UV-induced PI-3 kinase and attenuated UV-induced Akt and p70 S6-K, both downstream effectors of PI3 K (Nomura et al. 2001b). Studies with oral administration of green tea or caffeine to mice reported chemopreventive effects on UV induced carcinogeneses mediated through sitmulation of UV-induced increases in the number of p53 positive cells, $p21^{Waf1/cip1}$ positive cells and apoptotic sunburn cells (Lu et al. 2001).

Investigators have developed a 10% (w/w) EGCG formulation in Hydrophilic ointment USP for topical application. An intradermal uptake of 19% and 0.9% of the applied dose was evident in the mouse and human skin, respectively, while transdermal penetration was observed only in the mouse skin (Dvorakova et al. 1999). The 10% EGCG formulation was used in a phase I clinical trial to assess safety and the sun protection factor (SPF). An SPF of 3.6 was recorded for this ointment, applied to buttock skin. No systemic toxicities with topical application to the arms were seen in 19 participants that completed the study.

However, 42% of the participants reported moderately severe skin reaction and histological evaluation corroborated the clinical findings.

The chemopreventive activity of aspirin and sodium salicylate were investigated in a UVB-induced NMSC hairless SKH-1 mouse model (Bair et al. 2002). While sodium salicylate significantly inhibited UVB-induced tumor formation, aspirin had only a moderate effect. The protection supplied by sodium salicylate appears to be in part due to its sunscreen effect, which was demonstrated by the reduction of thymine dimers in the epidermis of mice treated with sodium salicylate. Aspirin was unable to prevent dimer formation (Bair et al. 2002).

In vitro studies revealed that a derivative of nordihydroguaiaretic acid (NDGA), tetra-O-methylnordihydroguaiaretic acid, inhibited growth of several tumor cell lines, including a melanoma line where there was morphologic evidence of apoptosis. This compound also inhibited the synthesis of DNA and caused cell cycle arrest in $G_0/G_1$ and $G_2/M$ phases of the cell cycle. Growth inhibitory effects of this compound were also exhibited in vivo (Lambert et al. 2001). Bowden and colleagues have also identified NDGA as an inhibitor of UVB-induced c-Fos and AP-1 transactivation by inhibiting the PI-3 kinase signal transduction pathway (Gonzales and Bowden 2002).

A potential chemopreventive agent for melanoma, apomine, has been studied in a clinical and preclinical setting. Apomine is a bisphosphonate ester that has been reported to activate the farnesoid X receptor, increase the rate of degradation of HMG-CoA reductase and induce apoptosis (Niesor et al. 1999). Apomine has been shown to inhibit the growth of many tumor cell lines, including those derived from leukemia, colon, liver, ovary and breast (Falch et al. 2000). Growth inhibition and apoptotic activity were compared to those of simvastatin, farnesol and 25-hydroxycholesterol, which all affect HMG-CoA reductase. Apomine was most like farnesol, a non-steriol regulator of cholesterol synthesis. In a phase I trial at the Arizona Cancer Center, it was demonstrated by plasma pharmacokinetics that a daily dose of 125 mg $m^{-2}$ of apomine was sufficiently bioavailable to levels used in in vitro studies that demonstrated activity against fresh human solid cancers. In preliminary studies in a TPras transgenic melanoma mouse model (Powell et al. 2002), apomine caused a 55% reduction in melanoma development induced by DMBA. In vitro studies with melanoma cell lines derived from the transgenic TPras mouse model and treated with apomine demonstrated a significant reduction in Ras detected in the membrane fraction (activated Ras). Apomine was also able to reduce UVR-induced Akt phosphorylation but had no effect on phosphorylation of ERK1/2 (Powell et al. 2002). A study in human melanoma cells found that apomine cytotoxicity to the cells was mediated primarly through a non-apoptotic pathway (Pourpak et al. 2005). In a phase I clinical trial at the Arizona Cancer Center, apomine expressed prolonged cancer stabilization in patients with metastatic melanoma and recurrent ovarian cancer with minimal or no toxicity (Powell et al. 2002). A topical formulation with apomine is in preparation for clinical trials in skin cancer. Investigators at the Arizona Cancer Center have developed the high performance liquid chromatography method to analyze apomine in topical cream formulations (Kuehl et al. 2006).

Another agent with potential chemopreventive activity in both melanoma and NMSC is perillyl alcohol (POH). POH is a cyclic monoterpene that reduces the amount of Ras and Ras-related proteins and has been reported to induce apoptosis. POH is found in the essential oils of numerous plants including citrus fruit, cherries and mint. Limonene

(a precursor of POH) has been demonstrated to reduce the incidence of spontaneous lymphomas in $p53^{-/-}$ mice and to inhibit the development of chemically-induced rodent mammary, liver, lung and forestomach tumors (Crowell 1999). Ras oncogene-induced mammary carcinoma development has also been inhibited by limonene (Gould et al. 1994). POH has demonstrated chemopreventive properties in several types of cancers, including liver cancer in rats (Mills et al. 1995), pancreatic cancer in hamsters (Stratton et al. 2000) and mammary tumors in rats (Haag and Gould 1994). POH and limonene as oral agents have also been used in clinical trials (Gould et al. 1994; Crowell 1999). Chemopreventive properties of topical POH have been demonstrated in a non-melanoma and a melanoma mouse model (Barthelman et al. 1998b; Lluria-Prevatt et al. 2002). In both models, topically applied POH significantly reduced the incidence of tumors. Investigators also reported that POH reduced detectable levels of Ras, inhibited the activation of Akt and MAPK and reduced UVR-induced reactive oxygen species in melanoma cells (Lluria-Prevatt et al. 2002). POH inhibited UVB-induced AP-1 transactivation in vitro and in vivo (Barthelman et al. 1998a). The mechanisms of action for POH identified thus far include inhibition of cell proliferation, induced tumor cell differentiation (Morse and Stoner 1993) and increased apoptosis (Mills et al. 1995). POH has been shown to inhibit protein isoprenylation in Ras (Hohl and Lewis 1995; Stayrook et al. 1998). Evidence of chemopreventive activity in mouse models and the suspected molecular targets for POH makes it an ideal compound for potential chemoprevention studies in melanoma and NMSC. For clinical studies, POH has been formulated into a topical cream (Gupta and Myrdal 2004). The formulation was found to be physically and chemically stable over a period of 1 year at 4° and 25°C. A phase IIa randomized, placebo controlled, double-blind trial of topical POH in sun damaged skin has been initiated at the Arizona Cancer Center in Tucson, Arizona to evaluate its chemopreventive activity in humans.

Future agents will most likely be identified by their mechanism of action. The selected agents will have specific targets such as those described earlier as important in the UV signaling pathways and carcinogenesis process of skin cancer development (Fig. 2). For p38 MAPK, there are inhibitors which are a group of polycyclic pyridinylimidazole compounds. SFK86002, a bicyclic pyridinylimidazole, first reported to inhibit LPS-stimulated cytokine production (Lee et al. 2000). Early reports indicated a role of cytokine inhibition as a potential mechanism for the potent anti-inflammatory activity of these compounds (Lee et al. 1988). Subsequently, SB203580 and other 2,4,5-triaryl imidazoles were prepared as a tool for finding the molecular target involved in cytokine regulation (Lee et al. 1993). Later discoveries indicated p38/CSBP as the molecular target of these compounds (Gallagher et al. 1997). One such compound, SB202190, inhibits p38 phosphorylation of myelin basic protein (MBP) while not effecting ERK or JNK MAP kinases. The compound also inhibits p38 phosphorylation of activating transcription factor 2 and blocks LPS-induced TNF and interleukin biosynthesis as well as inducing LDL receptors in vitro (2002). Investigators have used SB202190 to understand the mechanisms of UVB- and UVA-mediated p38 MAPK (Bachelor et al. 2005; Bachelor and Bowden 2004). Topical treatment to mouse epidermis with SB202190 resulted in a 84% decrease in UVB-induced AP-1 activation as well as COX-2 expression (Bachelor et al. 2005).

It has been demonstrated that topical application of the compound resveratrol demonstrates chemopreventive effects against multiple short term UVB exposures to the skin of

hairless mice by decreasing the UVB mediated upregulation of MAPKK and the 42 kDa isotope of MAPK (Reagan-Shaw et al. 2004). Resveratol is a naturally occurring antioxidant phytoalexin produced by some plants subsequent to injury or fungal infection and is found in red wine and grapes (Aziz et al. 2003, 2005). This agent has shown cancer chemopreventive effects in a variety of tumor bioassays (Aziz et al. 2003; Jang et al. 1997; Kapadia et al. 2002) and has been attributed to the "French Paradox" (Aziz et al. 2003; Kopp 1998; Sun et al. 2002). Early studies demonstrated that resveratrol inhibited chemically induced skin tumorgenesis in a mouse model (Jang et al. 1997). Most recently, Aziz and colleagues demonstrated that topical resveratrol was a chemopreventive agent in long term UVB exposure of a mouse skin carcinogensis model (Aziz et al. 2005). The untreated group resulted in SCC, Bowen's disease, invasive carcinomas in situ and AK. The resveratol treated group (pre- and post-treated) had a significant reduction in tumor incidence and the majority of the lesions were AKs suggesting that the agent was inhibiting the malignant conversion of AKs. The chemopreventive effects of resveratrol appear to be against modulation of cki-cyclin-cdk network and MAPK pathway (Reagan-Shaw et al. 2004). The activity of the agent appears to be through signaling pathways rather than a sunscreen effect (the treatment was also effective post UV irradiation) (Aziz et al. 2005). These studies suggest that the target for resveratol is the modulation of the expression and function of survivin. Survivin is a critical regulator of cell survival/death (Altieri 2001; Altieri and Marchisio 1999). Deregulation of survivin has been shown to inhibit melanoma tumor growth (Grossman et al. 2001) and found to prevent papilloma regression and promote conversion to SCC (Altieri 2003).

SP600125, an anthrapyrazole, is an inhibitor of JNK catalytic activity (Bennett et al. 2001). This inhibitor was identified in a high-throughput biochemical screen by using purified recombinant JNK2 and c-Jun. SP600125 demonstrated inhibitory activity consistent with the role of JNK in CD4+ cell activation and differentiation, CD14+ cell gene expression and in thymocyte death. SP600125 inhibits c-Jun phosphorylation in cells and also COX-2, IL-2, IFN-c, TNF-α, IL-10 and also MMP gene expression (Han et al. 2001). In vivo studies demonstrated that SP600125 inhibited LPS-induced TNF-α expression in mice (Bennett et al. 2001). SP600125 also prevented anti-CD3-mediated thymocyte apoptosis in a C57BL/6 mouse model (Bennett et al. 2001). In addition, this inhibitor of JNK blocked cell proliferation but did not kill CD4+ cells, resulting in a cytostatic effect on T cell proliferation. Although several anthrapyrazoles have been identified as chelators of DNA (i.e., doxorubicin), SP600125 did not exhibit characteristics of a strong interchelator of DNA in competitive binding assays. SP600125 also did not induce apoptosis (Bennett et al. 2001). Both compounds, the MAPK inhibitor, SB202190, and the JNK inhibitor, SP600125, were able to inhibit UVA-induced AP-1 and c-fos transactivation as well as c-fos expression in the HaCat cell line transfected with a luciferase reporter (Silvers et al. 2003).

Inositol hexaphosphate ($InsP_6$) is a direct inhibitor of PI-3 kinase in vitro (Huang et al. 1997a,b,c). This agent has also demonstrated inhibition of EGF-induced AP-1 activation and cell transformation of JB6 epidermal cells (Huang, 1997). $InsP_6$ also inhibits UVB-induced AP-1 and NF-jB transcriptional activity. This compound is similar in structure to a potent PI-3 kinase inhibitor, D-3-deoxy-3-fluoro-PtdIns (Powis et al. 1995).

LY294002 is a morpholino derivative of the broad-spectrum kinase inhibitor, quercetin. This compound is also an inhibitor of PI-3 kinase. This agent has been shown to cause

inhibition of UVB-induced COX-2 promoter activity and protein expression of COX-2 in human keratinocytes (Tang et al. 2001). Topical LY294002 treatment in SKH-1 mouse epidermis demonstrated a significant induction of UVB induced AP-1 activation as well as COX-2 expression (Bachelor et al. 2005). Studies using TPras transgenic melanomas in SCID mice demonstrated a reduction in invasion, correlated with a reduction in MMP2, when treated with LY294002 (Bedogni et al. 2004). Using the TPras mouse model for melanoma development, discussed earlier in this chapter, topical application of LY294002 resulted in a delay in melanoma development by 8 weeks (Bedogni et al. 2006). In addition treatment with LY294002 after tumors had developed in this model resulted in only a 17% progression compared to 93% progression in the control mice. There was a 67% partial regression and a 17% regression in the mice treated with LY294002. Most interesting of these studies is that a combination of LY294002 and U0126, a specific inhibitor of MEK 1/2 increased the effectiveness. In the topical combination treatment, 70–75% of the mice did not develop melanoma while the control group only contained 9% of mice that were melanoma-free at the end of the study. The response to these agents corresponds to increased apoptosis and decreased proliferation both in vitro and in vivo as well as a reduction in tumor angiogenesis. These studies support the role of PI-3 kinase/Akt and Raf/MAPKK pathways as important in the development of melanoma. In addition the studies point to a potential for using LY294002 or other PI-3 kinase inhibitor as a topical chemopreventive agents in both melanoma and non-melanoma and a combination of LY294002 with U0126 in melanoma. Finally, treatments of these compounds in animal models demonstrated no systemic toxicities or skin irritations.

Three specific inhibitors of EGFR tyrosine kinase, PD153035, AG1478 and ZD1839, may be potentially useful as chemopreventive agents. PD153035 is a 4-anilinoquinazoline compound that acts via competitive binding at the ATP site with the RGF receptor (Fry et al. 1994). AG1478 is a member of a family of tyrosine phosphor kinase inhibitors called tyrophostins (Gazit et al. 1996), which were designed to mimic the tyrosine substrates. Investigators have shown that these inhibitors (Zhang et al. 2001) can block UVA-induced EGFR signaling. ZD1839 (Iressa; AstraZeneca Pharmaceuticals) is another inhibitor of EGFR, which could be considered for topical formulation development as a chemoprevention agent. ZD1839 has been shown to inhibit activation in a variety of human skin cell types in vivo subsequent to oral therapy (Albanell et al. 2002). In association with the EGFR inhibition, MAP kinase activation and keratinocyte proliferative rates decreased and an increase in the apoptotic index also occurred during therapy. ZD1839 is a substituted anilinoquinazoline that selectively inhibits EGF-stimulated tumor cell growth and blocks EGF-stimulated autophosphorylation in tumor cells (Wakeling et al. 2002). Clinical trials with oral ZD1839 have shown this compound to provide well-tolerated anti-tumor activity in patients (Wakeling 2002; Lorusso 2003).

As discussed earlier, BRAF is a potential molecular target for the chemoprevention of melanoma. BAY 43–9006 is a potent inhibitor of Raf kinase (Lyons et al. 2001). Oral administration of this compound has shown significant activity in four different human tumor types including colon (Gianpaolo-Ostravage 2001), pancreatic, lung and ovarian tumors carried out in xenograft models. Clinical testing of this compound in cancer patients began in July 2000 (Strumberg et al. 2001). Preliminary clinical data reported the compound to be well tolerated. At least 37% of patients in the initial study had stable disease

lasting longer than 12 weeks. This compound could be a promising agent for chemoprevention specifically for melanoma.

One compound which has shown an induction of apoptosis via the activation of the Bcl-2 family in human keratinocytes is sanguinarine (Adhami et al. 2003). This agent is derived from the root of Sanguinaria Canadensis and is also found in poppy and Fumaria species (Shamma and Guinaudeau 1986). The agent has been found to act as an antioxidant (Vavreckova et al. 1994) as well an antimicrobial (Mitscher et al. 1978) and anti-inflammatory compound (Mandel 1988). Sanguinarin has demonstrated potential as an chemopreventive agent in UVB irradiated human keratinocytes (Reagan-Shaw et al. 2006) and resulted in a decrease in UVB mediated skin edema, skin hyperplasia and infiltration of leukocytes in mice treated with topical sanguinarine (Ahsan et al. 2007).

A review by Juge and colleagues describes the chemopreventive effects of sulforaphane, a natural extract from broccoli sprouts (Juge et al. 2007). The chemopreventive properties include inhibition of phase 1 cytochrome P450 enzymes, induction of phase 2 metabolism enzymes, antioxidant functions through increased tissue GSH levels, apoptosis-inducing properties, induction of cell cycle arrest, anti-inflammatory properties and inhibition of angiogenesis. Topical application of sulforaphane demonstrated an inhibition of skin tumorigenesis when applied to a mouse model system that uses DMBA as an initiator and TPA as the promoter of tumorigenesis (Gills et al. 2006). The topical application of sulforphane was effective during the promotion phase where it caused a significant decrease in both the percent of mice with tumors and in tumor multiplicity. In addition the agent inhibited TPA-induced ODC activity in the mouse skin which has also been demonstrated in mouse epidermal cells in culture (Lee et al. 1999). As discussed previously in this chapter, UVB can induce the activation of AP-1 and it is suggested that AP-1 plays a critical role in UVB-induced skin tumor development (Barthelman et al. 1998a; Huang et al. 2000). Investigators have demonstrated that sulforaphane can inhibit UVB-induce AP-1 activation in human keratinocytes by inhibiting AP-1 DNA binding activity (Zhu and Bowden 2004). Another group of researchers have used a hairless mouse model to demonstrate that topical sulforaphane substantially inhibited skin carcinogenesis induced by UVR (Dinkova-Kostova et al. 2006). This study showed a 50% reduction in tumor burden, incidence and multiplicity in animals which received topical sulforaphane after the completion of a 20 week regimen of UV irradiation. A dose escalation safety study in healthy humans revealed no adverse reactions with doses up to 340 nmol of topical sulforaphane and showing an increase in NAD(P)H:quinine oxido-reductase 1 (NQO1) therefore demonstrating an induction of phase 2 response in humans (Dinkova-Kostova 2007). The evidence presented here provides evidence that topical sulforaphane should be pursued as a potential chemoprevention for skin cancer.

Additional potential chemopreventive agents for skin cancer include curcumin, which induces apoptosis in human melanoma cells through a cell membrane-mediated mechanism independent of the p53 pathway by induction of the Fas receptor and activation of caspase-8 (Bush et al. 2001); and topical apigenin which has been shown to be effective in preventing UV-induced mouse skin tumorigenesis by inhibition ornithine decarboxylase (ODC) activity (Birt 1997).

Meyskens et al. (Meyskens et al. 2004) present a review of studies that suggest that ROS may be central to the pathogenesis of melanocyte transformation and melanoma

progression. They suggest a critical early pathogenic event is the change of anti-oxidant to pro-oxidant melanin, the pigment produced by melanocytes. Once the melanin is oxidized by ROS generated by UV, an accumulation of metals occurs and the antioxidant response is depleted, the build-up of ROS occurs. This, in turn, leads to melanosomal damage, DNA mutations, transcription activation and enhancement and activation of an anti-apoptotic (drug resistant) phenotype of melanocytes. Chemoprevention of melanoma within the context of this etiological hypothesis may involve the early use of antioxidants.

Other UVR-induced oxidative stress studies are emerging to understand the signaling pathways leading to antioxidant response elements (ARE) for its potential in developing skin cancer chemoprevention strategies. These investigations have found that UVB irradiation can interrupt the signaling of ARE through the JNK pathway in human keratinocytes (Zhu et al. 2006). Additional findings include UVB induced glutathione depletion in cultured keratinocytes through the caspase cascade (Zhu and Bowden 2004). Therefore targeting events in the JNK or caspase pathways may be suitable as a chemopreventive measure to allow the signaling of the ARE during UVR exposure that causes damage in skin cells that can develop into skin cancer.

Development of a group of novel agents for skin photoprotection called quencher of photoxcited states (QPES) (Wondrak et al. 2005) may also be included as strategies of chemoprevention. These compounds directly antagonize the potentially damaging excited state of skin chromophores and molecular oxygen which cause damage to cellular targets leading to skin photoaging or photocarcinogenesis. These compounds suppress skin photoxidative damage upstream of ROS formation. Investigators suggest that this compounds be used in combination with antioxidants and sunscreens for a complete form of photoprotection. With a thorough screening process in place for QPES-agents, Wondrak and colleagues (Wondrak et al. 2005) suggest proline ester derivatives to be optimized for topical application in the skin.

## 11.5 Conclusion

Skin cancer is a major health problem in the US as well as in countries such as Australia. With high health care costs, increasing incidence, limited treatments, and a significant loss of life specifically for melanoma, prevention of this disease is imminent. Primary prevention strategies focus on an avoidance of sun exposure and the use of sunscreen compounds. Significant advances in molecular biology in combination with pharmaceutical developments have opened the door for research in the field of chemoprevention. For skin cancer, the formulation of a UV-induced signal transduction pathway (Fig. 2) that identifies important molecules involved in the carcinogenesis process has provided molecular targets for the development of target-specific agents. This pathway has been developed by the use of animal and cellular model systems of skin carcinogenesis. These targets include AP-1 and COX-2, as well as upstream targets such as EGFR, PI-3 kinase, MAPK, JNK and Raf. Ongoing and future clinical trials will evaluate agents that act specifically to block molecules that are altered early in the development of skin cancer. These agents will most likely be delivered in a topical formulation using technology (e.g. prodrug development)

that allows for maximum epidermal delivery with minimal systemic toxicity. The combination of several chemoprevention agents working in a synergistic fashion in these topical formulations will provide a promising strategy for the prevention of skin cancer.

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# Colorectal Cancer Prevention

# 12

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Adenocarcinomas of the colon and rectum (colorectal cancer, CRC) are malignant epithelial neoplasms. A polyp is a localized lesion that projects above the surrounding mucosa. Adenomatous polyps (adenomas) are benign neoplasms that arise from colorectal glandular epithelium. In the United States (US), colorectal adenoma (CRA) prevalence is approximately 25% by age 50 and rises to around 50% by age 70. The histological hallmarks of a CRA are altered glandular architecture and dysplasia of the epithelium. The great majority of CRCs develop from CRAs in a process called the adenoma–carcinoma sequence. This process may take from years to decades for the earliest CRA to progress to CRC (Leslie et al. 2002). A CRA progresses to become a CRC when the dysplastic cells invade through the muscularis mucosa. While most CRCs develop from CRAs, fewer than 10% of CRAs ever progress to CRC. Hyperplastic colorectal polyps are histologically distinct from CRAs, occur most frequently in the rectum and sigmoid colon, are not neoplastic and do not progress to CRC. Besides CRAs, the inflammatory bowel diseases, ulcerative colitis (UC) and Crohn's disease of the colon (Crohn's colitis), predispose to CRC.

In 5% or fewer of cases, predisposition to CRC is inherited as an autosomal dominant or other Mendelian disorder, often with associated predispositions to benign or malignant tumors of other organs (Kinzler and Vogelstein 1996; Lindor 2004). Familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) account for most of the autosomal dominantly inherited CRC cases. With the exception of the chromosomes that determine sex, a diploid nucleus contains two very similar versions of each chromosome, one from each parent. It follows that each diploid nucleus carries two versions (alleles) of each autosomal gene. In FAP, the inherited, germline abnormality (mutation) is in the *Adenomatosis Polyposis Coli* (*APC*) gene, a tumor suppressor gene. In HNPCC, the germline abnormality is in one of the mismatch repair (MMR) genes, whose products coordinate to repair defective DNA. Colorectal tumorigenesis does not occur in individuals with germline FAP or MMR gene mutations while the other, wild-type allele of the mutated gene functions normally. Tumorigenesis occurs when an inactivating mutation occurs in the wild-type FAP or MMR gene in the somatic, colorectal epithelial cell from which the neoplasm arises.

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Most CRC cases arise outside the context of one of the inherited CRC syndromes and are termed sporadic. It is now clear that the molecular pathogenesis of most sporadic CRCs involves either the *APC* or MMR gene pathway. The difference between familial and sporadic cases is that in the latter, both "hits" to the *APC* or MMR alleles are somatic and occur in the colorectal epithelial cell where tumorigenesis originates. But *APC* or MMR malfunction alone does not cause malignant transformation. A developing neoplasm must acquire sequential malfunction of multiple genes, usually four to six or more, before progression to invasive malignancy occurs (Hanahan and Weinberg 2000; Hahn and Weinberg 2002).

CRCs evolve through distinct genetic pathways involving genetic instability, which is the driving force for tumor development (Lengauer et al. 1998). Three genetic instability pathways have been identified in CRC. They are called chromosomal instability (Rajagopalan et al. 2003), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP). Chromosomal instability is the tumorigenic mechanism in FAP and in approximately 85% of sporadic CRCs that develop as a result of losses of both *APC* alleles and other tumor suppressor genes (Vogelstein et al. 1988). HNPCC and the remaining 15% of sporadic CRCs develop because of MSI that results from inactivation of the DNA MMR system (Ionov et al. 1993). Clusters of cytosine-guanosine pairs, termed CpG islands, in the promoter regions of many genes are prone to age-related methylation. Such "hypermethylation" can lead to gene silencing. In a proportion of CRCs, functional loss of certain tumor suppressor genes is caused by CpG island methylation and silencing rather than mutations (Toyota et al. 1999).

## 12.1 Epidemiology

CRC is the second most frequent fatal malignant neoplasm in the US. It is estimated that in 2008 there were 148,810 new cases and 49,960 deaths from the disease (Jemal et al. 2008). Sporadic CRC is uncommon below the age of 50 years (Cooper et al. 1995). The incidence rises to 159 per 100,000 by age 65–69 and 387 per 100,000 for those over the age of 85. CRC affects men and women at approximately equal rates (Jemal et al. 2008). Rates are highest among African Americans, with intermediate rates observed among Whites, Asians, Pacific Islanders and Hispanics (Ward et al. 2004). Low incidence rates have been observed among American Indians and Alaska Natives.

Worldwide, the incidence of CRC varies 20-fold, with the highest rates reported in the US and other western countries and the lowest in India (Bingham and Riboli 2004). The incidence is increasing rapidly in some countries where previously rates were low. For example, 40 years ago, CRC was rare in Japan whereas incidence in Japanese men at ages 55–60 years is now twice that of men in the United Kingdom (UK) (Bingham and Riboli 2004).

The risk for developing CRC increases with migration from a low-risk area to a high-risk area (Potter 1999). This was seen in Japanese migrants to the US and Hawaii in the 1950s and 1960s, whose risk became greater than native Japanese living in Japan. Furthermore, offspring of the Japanese migrants developed risks similar to US white populations. Similar increases in risk were seen in Europeans that migrated to Australia and in Jews that migrated to Israel from North Africa.

Approximately 50% of CRCs occur in the rectum and sigmoid, 25% in the cecum and ascending colon, 15% in the transverse colon and 10% in the descending colon. This distribution pattern changes with age. There is an increased propensity for proximal neoplasms with increased age. At age 65–69 years, 36% of CRCs occur proximal to the splenic flexure but by age 85 and older, 50% are proximal to the splenic flexure. This has implications for the optimal screening modality in CRC prevention programs (Cooper et al. 1995).

## 12.2 Risk Factors

The wide range of CRC incidence rates around the world is attributed largely to dietary differences (Potter 1999; Mason 2002; Bingham and Riboli 2004). Diets rich in foods from plant sources, low in saturated fat and red meat, and low in calories and alcohol (Giovannucci et al. 1995) are generally considered protective against CRC. Conversely, consumption of a Western diet high in calories, animal fat and refined carbohydrates, and a high body mass index (BMI) combine to increase CRC risk. These lifestyle factors, also risk factors for type-2 diabetes, are associated with hyperinsulinemia, insulin resistance and elevated levels of insulin-like growth factors (IGFs), which are mitogenic in normal and neoplastic cells (LeRoith et al. 1995). Circulating IGF-1 (formerly called somatomedin-C) level is associated positively with CRC risk (Ma et al. 1999; Palmqvist et al. 2002) and elevated insulin production may predict CRC risk independently of body mass index and IGF-1 level (Ma et al. 2004). Most of the clinical and biochemical effects of acromegaly result from stimulation of IGF-1 production by unregulated growth hormone secretion. CRC risk is increased over twofold in acromegalics, presumably as a result of sustained elevations of IGF-1 level (Baris et al. 2002).

### 12.2.1 Physical Activity

The evidence that a high level of physical activity protects against colon cancer is convincing (Colditz et al. 1997; Friedenreich 2001). At low levels of activity, obesity may be an additional independent risk factor. Physical activity may protect against rectal (Slattery et al. 2003) as well as colon cancer but the evidence is less emphatic.

### 12.2.2 Family History of Colorectal Adenoma or Colorectal Carcionoma

As discussed above, the predisposition to CRC is autosomal dominantly inherited in kindreds with FAP or HNPCC. CRAs numbering in the hundreds or thousands usually develop during the second decade in individuals with FAP. Without prophylactic panproctocolectomy the likelihood of malignant transformation approaches 100%. An attenuated form of FAP (AFAP) has been described (Spirio et al. 1998). AFAP is characterized by

lifetime accumulation of 10–20 rather than hundreds or thousands of CRAs and a later age of onset than unmodified FAP (see Chap. 5). Gene penetrance in HNPCC is 75–80%. Diagnosis of CRC in these patients is rare before the age of 25 years, occurs on average at age 45 years and has occurred in 70–80% of those affected by age 70 years. The majority of CRCs are in the proximal colon in HNPCC patients, who usually develop no more than tens of CRAs and very rarely more than 100.

FAP and HNPCC account for less than 5% of CRC cases, but familial clustering outside the context of well-characterized CRC family syndromes is considerably more common so that a familial component contributes to 20–30% of cases (Grady 2003). Risk for CRC can be increased up to eightfold in individuals with one or more first-degree relatives (parents, siblings or children) who have had CRC (Grady 2003). The magnitude of risk depends on the age at diagnosis of the index case and the number of affected relatives.

### 12.2.3 Inflammatory Bowel Disease

Ulcerative colitis (UC) and Crohn's disease comprise the chronic idiopathic inflammatory conditions termed inflammatory bowel disease. Patients with inflammatory bowel disease are thought to be at increased risk for CRC. The evidence of this connection is more robust for UC than Crohn's disease (Jess et al. 2004). The major determinants of CRC risk in patients with UC are the duration and extent of disease. Risk is not elevated for the first 8–10 years after diagnosis but thereafter increases by 0.5–1.0% yearly (Munkholm 2003). Patients with pancolitis (total colitis as judged by appearances at colonoscopy) are at greatest risk and this is corroborated by evidence that the severity of inflammation due to UC is an important determinant of risk for CRC (Rutter et al. 2004). Primary sclerosing cholangitis complicating UC compounds the risk for CRC, leading to cumulative rates as high as 50% after 25 years (Jayaram et al. 2001).

CRC-related mortality rates in patients with UC reported in recent years have been lower than in older reports. This apparent improvement reflects several factors. Most large recent reports are population-based studies as opposed to many of the earlier studies, which reported selected series of patients with mostly severe disease from specialist referral centers. Some of the lowest reported rates are from countries, such as Denmark, or regions where prophylactic colectomy rates are high. It is also possible that the now widely applied use of prolonged aggressive therapy with 5-aminosalicylic acid has chemopreventive benefit against progression to CRC. Recent population-based studies suggest an overall normal life expectancy in patients with UC (Winther et al. 2003) and a modest reduction of life expectancy in younger patients with Crohn's disease that is not attributable to CRC (Card et al. 2003).

### 12.2.4 Other Risk Factors

Risk of CRC is increased by 100–7,000 times in patients with a ureterosigmoidostomy (urinary diversion procedure by surgical placement of the ureter into the colon) (Woodhouse

2002). The most reliable estimate of excess risk is probably near the lower end of this range. The latency period is long with an average of 20 years but shorter periods have been reported. Nitrosamines generated from the diverted urine are thought to be important etiologic factors.

Asymptomatic CRAs and CRCs have been reported in patients diagnosed with *Streptococcus bovis* infective endocarditis. Although the frequency of this association is controversial (Gonzalez-Juanatey et al. 2003), screening colonoscopy is usually recommended for these patients. A similar association has also been reported with *Streptococcus agalactiae* infection.

Single nucleotide polymorphisms (SNPs) in genes other than *APC* or those of the MMR family are increasingly recognized as determinants of risk for developing CRAs and CRC. For example, a SNP affecting the expression of *ornithine decarboxylase* (*ODC*), a downstream gene in the *APC*-dependent pathway to CRC, is associated with risk of CRA recurrence (Martinez et al. 2003).

## 12.3 Screening and Early Detection

Currently, the US Preventive Services Task Force recommends approximately 50 different preventive services, with CRC screening being one of the most important. In an analysis of these 50 services, it was estimated that CRC screening by fecal occult blood testing (FOBT), flexible sigmoidoscopy or the combination (at appropriate intervals) when delivered to 100% of the target population would result in 225,000–450,000 quality-adjusted life-years (QALY) saved at a cost of US $12,000–$18,000 per QALY saved (Coffield et al. 2001). Of the 50 preventive services, only nine others, including childhood and influenza vaccinations, tobacco cessation counseling, and cervical cancer screening, achieved an equal or greater QALY savings and cost-effectiveness rating. (Please refer to Chap. 2 for a more detailed discussion of QALYs.)

The rationale for CRC screening is based on two premises. First, the proportion of early-stage CRCs with favorable prognosis is greater for CRCs detected by screening than for those diagnosed after symptoms have developed (Niv et al. 2002; Whynes et al. 2003). Second, most CRCs develop from CRAs and most screen-detected CRAs can be removed endoscopically by polypectomy. The observed subsequent incidence of CRC is reduced by as much as 90%, compared to the predicted incidence, following CRA polypectomy (Winawer et al. 1993; Citarda et al. 2001). Thus, the evidence to support population-wide CRC screening is compelling.

An individual's risk for developing CRC is considered as either average or increased. Groups at increased risk include: (1) patients with a past history of CRA or CRC; (2) patients with inflammatory bowel disease; (3) members of FAP or HNPCC kindreds; and (4) individuals with one or more first-degree relatives who have had CRAs or CRCs. The American Cancer Society and other authorities agree that average-risk men and women age 50 years and over should adopt one of the following screening strategies (Winawer et al. 2003; Smith et al. 2004):

1. Annual FOBT; or
2. Flexible sigmoidoscopy every 5 years; or
3. Annual FOBT and flexible sigmoidoscopy every 5 years; or
4. Double contrast barium enema every 5 years; or
5. Colonoscopy every 10 years

A problem affecting decisions on CRC screening policy is that FOBT is the only tool for which supportive evidence in the form of statistically significant outcomes of randomized controlled trials (RCTs) exists. Evidence for the other strategies comes only from case-control studies or mere conjecture and is, therefore, less secure. Another major problem is delivery. Screening rates among Americans are unacceptably low. The highest CRC screening rates are among Whites (41% for sigmoidoscopy and 34% for FOBT), the lowest are among Hispanics (28% for sigmoidoscopy and 21% for FOBT) and rates for African Americans and American Indians/Alaskan Natives are intermediate (BRFSS 2002). The lowest rates of screening occur among uninsured individuals, with 10–15% for FOBT and 15–20% for flexible sigmoidoscopy. Furthermore, individuals with lower levels of education have lower rates of screening: 29% for sigmoidoscopy among people with 11 or fewer years of education as opposed to 46% among those with 13 or more years of education.

### 12.3.1 Fecal Occult Blood Test (FOBT)

Much of the published experience with FOBT has come from Hemoccult guaiac-based stool tests, which detect the peroxidase activity of heme and other stool peroxidases. The American Cancer Society and other authorities in the US recommend annual FOBT without rehydration (Winawer et al. 2003; Smith et al. 2004). To reduce test inaccuracy, patients are asked to follow certain guidelines for several days before and during stool collection. They should avoid aspirin or other non-steroidal anti-inflammatory drugs, vitamin C and iron supplements, and red meat. The evidence-based protocol for FOBT CRC screening involves the patient sampling two separate regions of three consecutive spontaneously evacuated stool specimens. Each Hemoccult card has two windows, one for each of the regions sampled from a single stool, giving a total of six windows for a complete FOBT. For optimum quality control, cards should be sent for development to an appropriately monitored laboratory. When this is done, cards are usually not read until several days after defecation and sampling of the stool. FOBT positivity is recorded if a positive reaction is seen in one or more of the six windows to which stool was applied in a complete test. Structural evaluation of the rectum and colon, usually by total colonoscopy, is indicated in all patients with a positive FOBT.

The delay between the time of applying stool to FOBT cards and application of developing reagents in the laboratory further compromises an already insensitive test. Some sensitivity can be retrieved by card rehydration before applying the developing reagent but this drastically reduces test specificity, thereby leading to an unacceptable increase in the number of negative colonoscopies. The current unequivocal recommendation is that guaiac-based cards should not be rehydrated before development.

Besides rehydration, various FOBT modifications have been made to improve test performance. Hemoccult SENSA is a more sensitive guaiac-based test than the original Hemoccult and the more recent Hemoccult II version of this test. More expensive immunochemical tests that are specific for human haemoglobin have been introduced to improve specificity. These include HemeSelect, and the more recent InSure and FlexSure occult blood tests.

Longitudinal RCTs of FOBT have not included a criterion standard examination, such as a "gold-standard" colonoscopy. A few studies have compared performance of a single three-card application of FOBT among asymptomatic subjects to findings at a contemporaneous colonoscopy or air-contrast barium enema. Under these circumstances, the sensitivity of an unrehydrated FOBT for invasive CRC is approximately 30% (Ahlquist et al. 1993). In other words, FOBT is negative in two-thirds or more of patients who undergo a structural evaluation of the rectum and colon shortly after a positive FOBT.

Nonetheless, multiple-card hemoccult FOBT at 1- or 2-year intervals, respectively, has been shown to reduce CRC incidence and mortality, by 17–20% (Mandel et al. 2000) and 21–33% (Mandel et al. 1993; Mandel et al. 1999). FOBT-related reductions in CRC incidence and mortality occur as a result of the diagnostic and therapeutic interventions implemented because of a positive FOBT. It is noteworthy that in the study reporting the largest (33%) reduction in CRC mortality, FOBT was performed annually and greater than 80% of hemoccult cards were rehydrated before development (Mandel et al. 1993). Contributions to FOBT sensitivity and specificity from restrictions of medications and diet before and during stool collection have not been evaluated in a RCT. Likewise, performance characteristics of Hemoccult SENSA® and the immunochemical FOBTs have not been assessed in randomized trials (Young et al. 2003).

Physicians often perform a digital rectal exam (DRE), which is appropriate, but then smear stool from the gloved finger used for the DRE on a FOBT card, which is developed and read immediately in the office without quality control. Performance of FOBT in this manner is inappropriate and has never been evaluated rigorously. However, from indirect evidence the sensitivity and specificity of FOBT performed this way are so inferior that the practice cannot be supported. Fewer than 10% of CRCs arise within reach of the examining finger at DRE (Winawer et al. 1997).

### 12.3.2 Flexible Sigmoidoscopy

A 60-cm flexible endoscope is used for screening sigmoidoscopy. Flexible sigmoidoscopy is an office-based procedure, usually performed by primary care physicians or their non-physician assistants (Schoenfeld et al. 1999) without intravenous conscious sedation. An enema is administered immediately before the procedure. Complete examination of the rectum and sigmoid colon is routinely accomplished by appropriately trained practitioners but insertion of the flexible sigmoidoscope proximal to the junction of the sigmoid and descending colon is often not achieved. Authorities in the US recommend colonoscopy for all patients with any adenoma at screening sigmoidoscopy and repeat flexible

sigmoidoscopy 5 years after a negative examination. Once-per-lifetime screening flexible sigmoidoscopy for colorectal cancer is being assessed in a nationwide program in the UK (Atkin et al. 1993). Prevalence of distal adenomas and CRCs in an interim report of the UK trial was 12.1% and 0.3%, respectively (Atkin et al. 2002). In this trial, small polyps were removed during screening and colonoscopy was undertaken if high-risk polyps (three or more adenomas, 10 mm or greater in diameter, villous, severely dysplastic, or malignant) were found.

Case-control studies indicate that mortality from CRCs within reach of the instrument may be reduced by 60–80% as a result of therapy implemented for findings at flexible sigmoidoscopy (Newcomb et al. 1992; Selby et al. 1992). The disadvantage of screening flexible sigmoidoscopy is failure to identify proximal CRAs and CRCs, which are accessible only with the colonoscope. A proportion of these proximal lesions are identified at colonoscopies performed because of distal CRAs or CRCs found at flexible sigmoidoscopy. Adenomas of at least 10 mm in diameter and all those with villous histology or high-grade dysplasia are termed "advanced." Advanced adenomas are those at greatest risk for progression to CRC. New (metachronous) adenomas can develop in the years after adenoma polypectomy or CRC resection. Patients from whom advanced adenomas or CRCs were removed are those most likely to develop metachronous CRAs or CRCs. Thus, advanced adenomas as well as early-stage CRCs are crucial target lesions for CRC screening programs. From 46 to 52% of patients with proximal advanced adenomas or CRCs do not have synchronous distal CRAs or CRCs (Imperiale et al. 2000; Lieberman et al. 2000). The overall sensitivity of flexible sigmoidoscopy for diagnosing advanced adenomas and CRCs, using colonoscopy as the criterion standard, is estimated at 70–80% (Pignone et al. 2002).

The recommendation that a repeat screening flexible sigmoidoscopy need not be performed until 5 years after a negative examination is based on evidence of a low yield of advanced lesions at the second procedure. In one study, adenomas were reported in 6% of screened individuals at a second examination 5 years after the first, but none had CRCs or advanced adenomas (Rex et al. 1994). In another study, the interval between examinations was 3 years. CRAs or CRCs were found in 3.1% of patients at the second examination, including 0.8% with advanced adenomas or CRCs (Schoen et al. 2003).

Combining annual FOBT and flexible sigmoidoscopy every 5 years is an accepted screening strategy (Winawer et al. 2003; Smith et al. 2004). In years that flexible sigmoidoscopy is due, the annual FOBT should be done first; if positive, the patient is referred for colonoscopy and should not undergo an unnecessary sigmoidoscopy as well. The combination of FOBT and sigmoidoscopy been evaluated in neither randomized controlled trials nor case-control studies. Combined once-only FOBT and a surrogate for flexible sigmoidoscopy were evaluated in a study of screening colonoscopy (Lieberman and Weiss 2001). FOBT was performed before colonoscopy in all patients and those distal lesions identified within 60 cm of the anal verge at colonoscopy, the surrogate lesions, were designated as accessible by flexible sigmoidoscopy. FOBT was negative and distal, sigmoidoscopy-accessible CRAs or CRCs were absent in 24% of patients with proximal advanced CRAs or CRCs. Without colonoscopy, advanced proximal lesions would have been missed in these patients.

### 12.3.3 Barium Enema

The double contrast barium enema (DCBE) is a radiologic test in which barium and air are introduced into the rectum and colon. Radiographs are taken with the patient in various positions and mass lesions (CRAs or CRCs) are identified as projections outlined by a thin layer of barium against a dark background of air contrast. Patients with DCBE appearances of a polyp or cancer should undergo colonoscopy. Repeat DCBE in 5 years is recommended after a negative test.

DCBE is inferior to optical colonoscopy for detection of CRAs. In a study comparing the two procedures, approximately 50% of CRAs over 6 mm that were seen at colonoscopy were diagnosed by DCBE (Winawer et al. 2000). No randomized trials have been conducted to determine if DCBE reduces CRC incidence or mortality. DCBE is retained for the time being as a screening option because it offers the opportunity to visualize the entire colon. Its major application is for patients who are medically unsuitable candidates for invasive procedures. Virtual colonoscopy is likely to replace DCBE before long as the non-invasive method of choice for structural evaluation of the colon.

### 12.3.4 Colonoscopy

The American Cancer Society and other authorities recommend optical colonoscopy, to be repeated in 10 years following a negative study (Winawer et al. 2003; Smith et al. 2004) as a primary screening tool. Careful bowel preparation with non-absorbable lavage fluid is essential before colonoscopy, which is performed under conscious intravenous sedation. Because of the sedation, patients must be accompanied after the procedure and are not allowed to drive or return to work until the following day.

Assessment of the accuracy of optical colonoscopy is problematic because it is considered the criterion standard for other CRC screening modalities. To tackle this problem, the miss rate of colonoscopy was determined from findings at same-day, back-to-back colonoscopy performed by different endoscopists (Rex et al. 1997). Sensitivity for large adenomas was 90% and for small adenomas (less than 10 mm) was 75%. It is reasonable to assume that sensitivity for CRC is at least 90%. Studies reporting 90% or similar rates of sensitivity for detecting advanced colorectal lesions have almost invariably been conducted in specialist centers (Rex et al. 1997). It is unclear whether similar accuracy prevails in the community settings where most colonoscopies are performed.

There are no studies evaluating whether screening colonoscopy alone reduces the incidence or mortality from CRC but indirect evidence suggests substantial benefit. Estimates from the National Polyp Study were that up to 90% of CRCs could be prevented by regular colonoscopic surveillance examinations (Winawer et al. 1993). In a study of cost-effectiveness using models based on multiple assumptions, it was estimated that colonoscopic screening every 10 years would reduce CRC incidence and mortality by 58 and

61%, respectively (Frazier et al. 2000). These estimates of the benefits of screening optical colonoscopy await more rigorous confirmation.

Optical colonoscopy has several disadvantages as a primary screening tool. The obligatory bowel preparation is unpleasant and patients may experience discomfort during the procedure. Costs and logistical challenges of providing colonoscopy for the whole population over the age of 50 years are daunting. Complications can occur, the most serious being bowel perforation, which necessitates emergency abdominal surgery and can very rarely be fatal. Perforation rates due to colonoscopy vary quite widely. In the Veterans Administration Cooperative Study of 3,196 patients undergoing screening colonoscopy there were no perforations (Nelson et al. 2002). In a 5% sample of Medicare beneficiaries from regions of the US covered by the Surveillance, Epidemiology, and End Results (SEER) Program, there were 77 perforations after 39,286 colonoscopies for a rate of 1.96 of every 1,000 procedures (Gatto et al. 2003). In a prospective study of 9,223 colonoscopies performed over a 4-month period in three regions of the UK National Health Service, the perforation rate was 1.30 in 1,000 (Bowles et al. 2004).

### 12.3.5 History of CRAs or CRC

Management of patients who have had one or more CRAs removed at colonoscopy is based on findings from that procedure (Winawer et al. 2003). Those with three or more CRAs or an advanced CRA should have a surveillance colonoscopy at 3 years. Patients with one or two non-advanced CRAs should have the first follow-up colonoscopy at 5 years. If total colonoscopy was not performed preoperatively, patients should have the procedure 6 months after surgical resection of a CRC. For those who had a preoperative colonoscopy, a surveillance colonoscopy should be performed 3 years after surgery.

### 12.3.6 Inflammatory Bowel Disease

Patients with a history of UC or Crohn's colitis for more than 8–10 years should undergo colonoscopic surveillance (Winawer et al. 2003). Objectives are to remove polyps, sample elevated lesions (the dysplasia-associated lesion or mass, DALM) and obtain multiple biopsies over the full length of the colon. Biopsies should be read by pathologists with specialist expertise in gastroenterology for the presence and degree of dysplasia, which is used as an index of risk for future invasive cancer. The purpose of surveillance is to offer and perform prophylactic surgery before frank malignancy, which is often multi-focal, develops. Optimal care for these patients can best be provided by a multi-disciplinary team of experts. High-grade dysplasia is usually an indication for panproctocolectomy, which may also be indicated for low-grade dysplasia and for patients with UC or Crohn's colitis under other circumstances.

Surveillance programs have been shown to improve the survival of UC patients in a case-control study (Choi et al. 1993). All patients with UC or Crohn's colitis should undergo a first surveillance colonoscopy to re-assess the extent of colonic involvement

after 8–10 years of disease. The extent of disease and presence or absence of dysplasia will dictate the frequency of subsequent surveillance colonoscopy.

### 12.3.7 FAP and HNPCC Kindreds

Annual sigmoidoscopy starting at age 10–12 years to look for development of CRAs is recommended for children of a parent with FAP (Grady 2003). Clinical testing for *APC* germline mutations is widely available but should only be offered in conjunction with genetic counselling by qualified individuals. When a family s *APC* mutation has been identified, children born to the family can be tested for that mutation. If negative, they will not develop FAP and need not undergo surveillance sigmoidoscopy. Colonoscopy is recommended every 1–2 years starting at age 25, or 10 years earlier than the youngest age of colon cancer diagnosis in the family for children born to HNPCC kindreds (Winawer et al. 2003). Genetic testing can be offered to children at risk in HNPCC families for which the MMR gene mutation has been identified. If positive, the importance of surveillance for the gastrointestinal and other HNPCC-related cancers is emphasized to the patient and family. (Please refer to Chap. 5 for a complete discussion of genetic testing and the hereditary risk of cancer.)

### 12.3.8 First-Degree Relatives of People with CRAs or CRCs

People with a first-degree relative with CRC or CRAs diagnosed before 60 years of age or two first-degree relatives diagnosed with CRC at any age should have screening colonoscopy starting at age 40 years or 10 years younger than the earliest diagnosis in their family, whichever comes first (Winawer et al. 2003). Colonoscopy should be repeated every 5 years. People with a first-degree relative with CRC or CRAs diagnosed at the age of 60 years or older or two second-degree relatives with CRC should be screened as average-risk individuals, but from the age of 40 rather than 50 years.

### 12.3.9 Emerging Screening Tests

#### 12.3.9.1 Fecal DNA

With appropriate processing and the polymerase chain reaction (PCR), it is now possible to isolate and detect DNA from colorectal epithelial cells shed into the bowel lumen in stool specimens (Ahlquist et al. 2000). Proof of concept studies have shown that mutated DNA from *APC*, K-*ras* and other genes from neoplastic colorectal cells can be detected in this way (Ahlquist et al. 2000; Traverso et al. 2001). High throughput technology pioneered

by EXACT Sciences (Maynard, MA) has made it possible to process sufficient samples to begin trials of fecal DNA testing for CRC screening. In a recent study the PreGen-Plus™ fecal DNA test was performed on patients who underwent colonoscopy (Tagore et al. 2003). The sensitivity of PreGen-Plus™ for invasive CRC and advanced adenomas, respectively, in these patients was 64 and 57%. Preliminary results of a screening study comparing PreGen-Plus™ and FOBT to colonoscopy in 2,507 asymptomatic subjects at average risk for CRC were reported at a meeting but have not yet been published (Rex 2004). Sensitivity of PreGen-Plus™ and FOBT, respectively, for invasive CRC was 52 and 13% and for advanced adenomas was 15 and 11%. PreGen-Plus™ is commercially available. At this stage of development, the sensitivity of fecal DNA testing is intermediate between FOBT and colonoscopy but the cost is prohibitive. As the technology matures, this situation may change in favor of fecal DNA testing.

#### 12.3.9.2 Virtual Colonoscopy

Thin-section, helical computed tomography (CT) followed by off-line processing, a technique termed CT colonography or virtual colonoscopy, can yield high-resolution, three-dimensional images of the colon. For good resolution, at present the same bowel preparation as for optical colonoscopy must be administered, and the colon must be insufflated with air or carbon dioxide via a rectal tube. Expectations have been raised that the sensitivity and specificity of virtual colonoscopy may approach those of optical colonoscopy. Results from early studies comparing the two techniques lead to conflicting conclusions. For example, Pickhardt et al. reported almost identical sensitivities of around 90% for detection of adenomas at least 6 ml in diameter by virtual and optical colonoscopy (Pickhardt et al. 2003). In contrast, Cotton et al. reported a sensitivity of only 39% for detection of adenomas at least 6 mm in diameter by virtual colonoscopy compared to 99% sensitivity for the same lesions by optical colonoscopy (Cotton et al. 2004).

Various explanations have been proposed for these discrepant results. Patients in the Pickhardt study received double the usual volume of sodium phosphate for bowel preparation. This group used superior computer software for a virtual “fly-through” of the colon that has not been used by other groups. The sensitivity of optical colonoscopy achieved by the Cotton group is superior to most published reports and almost certainly not matched in routine daily practice.

Clearly, the role of virtual colonoscopy as a primary screening tool has not been finalized. Where available it is now the procedure of choice for structural evaluation of the colon in patients who are unsuitable for optical colonoscopy or in whom optical colonoscopy could not be completed. Disadvantages of virtual colonoscopy are the bowel preparation that is required, insufflation of the colon, which is uncomfortable, and the need for a second procedure, optical colonoscopy, to biopsy or remove lesions identified at a positive virtual procedure. It is agreed that patients with lesions at least 6 mm in diameter should be referred for optical colonoscopy. Consensus has not been reached on the management of patients diagnosed with small polyps at virtual colonoscopy. Most CRAs of diameter less than 6 ml in older average-risk individuals are of

trivial significance. Some advocate that patients in this category with one or two small adenomas diagnosed at virtual colonoscopy need not be referred for optical colonoscopy and polypectomy. The question of appropriate follow up if this "watchful waiting" approach is taken is unresolved. Options include optical colonoscopy or repeat virtual colonoscopy at intervals to be determined.

The field of CRC screening is evolving rapidly. Optical colonoscopy is the current criterion standard but whether it is feasible to aim for delivery of this procedure to the entire population over age 50 years is unclear. A major effort is under way to develop less invasive triaging tools, such as fecal DNA testing and virtual colonoscopy, with sufficient sensitivity and specificity that optical colonoscopy in the average-risk population could be restricted to people with a positive triaging test. Fecal tagging techniques using orally administered contrast agents are under development for the purpose of doing away with the need for bowel preparation before virtual colonoscopy. Successful development of fecal tagging would probably lead to more widespread use of virtual colonoscopy for CRC screening in the average-risk population.

## 12.4 Chemoprevention

The wealth of epidemiologic data implicating diet in colorectal carcinogenesis has provided the rationale for numerous efforts at reducing CRC risk by dietary and related interventions. The term chemoprevention is used to cover this topic, meaning the long-term use of oral agents to prevent colorectal neoplasms (Lamprecht and Lipkin 2003). The adenoma–carcinoma sequence and the fact that new (metachronous) CRAs develop in people from whom CRCs have been resected or CRAs removed have been exploited in the design of chemoprevention studies. Thus, randomized trials have been conducted in patients who have undergone removal of baseline CRAs by colonoscopic polypectomy. The subsequent rates of adenoma recurrence in the intervention and control groups are compared to determine the efficacy of the intervention.

### 12.4.1 Fiber

RCTs of wheat bran fiber supplements (13.5 g versus 2 g per day) (Alberts et al. 2000) and consumption of a diet low in animal fat and high in fiber, fruit and vegetables (Schatzkin et al. 2000) for 3–5 years failed to reduce adenoma recurrence rate. However, the validity of adenoma recurrence trials as surrogates for evaluating chemopreventive efficacy against CRC has been challenged on several grounds. Patients with baseline advanced adenomas have been in the minority in many CRA recurrence trials yet these are the patients in whom chemoprevention would be most applicable. It is possible that alternative factors influence recurrence in patients with advanced and non-advanced baseline CRAs. Many CRA recurrence trials have insufficient power to exclude significant benefit from the intervention(s)

in the subgroup with baseline advanced adenomas. In addition, only the early, initiation stage of the adenoma–carcinoma sequence is examined in CRA recurrence trials. Agents that do not inhibit CRA initiation and do not reduce CRA recurrence rate could, nonetheless, inhibit the more important events of adenoma progression and malignant transformation to CRC. Furthermore, the relatively short duration of CRA recurrence trials may be insufficient for anti-carcinogenic actions to take effect. Lastly, most participants in CRA recurrence trials are in their sixth decade or older. To be active, it is possible that chemopreventive agents must be introduced much earlier in life.

High intake of dietary fiber has been associated with decreased risk for CRAs (Peters et al. 2003) and CRCs (Bingham and Riboli 2004) in observational studies. It is possible that exercise and other healthy-lifestyle factors were important confounding variables in these studies.

The American Gastroenterological Association recommends a total fiber intake of at least 30–35 g of fiber per day (Kim 2000). Fiber should be from all sources, including fruits, vegetables, cereals, grains and legumes, because of possible interactions between fiber and anti-carcinogens present in fiber-rich foods.

### 12.4.2 Folate

In its chemically reduced form of tetrahydrofolate, folate is essential for cellular integrity (Lamprecht and Lipkin 2003). Folate plays important anti-neoplastic roles in the control of DNA methylation and DNA synthesis and maintenance. Folate consumption by participants in the Nurses' Health Study cohort was assessed by questionnaire. Relative risk for developing CRC was 0.25 for those with consumption in the highest quintile (more than 400 mcg per day) compared to those in the lowest quintile (200 per day or less) after 15 years of use (Giovannucci et al. 1998). Further analysis of these data showed that the greatest protection conferred by high folate consumption against developing CRC was in people with a family history of the disease (Fuchs et al. 2002). This suggests that inherited characteristics related to folate metabolism, the genotype, and influence risk for CRC, the phenotype.

### 12.4.3 Polymorphisms are Alternative Alleles of a Gene

There may be subtle functional differences among the respective protein products of different polymorphic alleles. An individual can be homozygous or heterozygous for polymorphic genes. If homozygous, both copies of the gene are the same allele. If heterozygous, each copy is a different allele. Multiple enzymes (gene products), such as 5, 10-methylenetetrahydrofolate reductase (*MTHFR*), are involved in the pathways of folate metabolism. A role for folate in preventing colorectal carcinogenesis has been shown in individuals homozygous for a common polymorphism of *MTHFR* (Lamprecht and Lipkin 2003).

### 12.4.4 Calcium

Calcium is an important micronutrient that controls a large number of intracellular processes with anti-neoplastic potential (Lamprecht and Lipkin 2003). Dietary calcium also binds to bile and fatty acids with the effect of curbing intestinal cell proliferation. Calcium supplements (1,200 mg daily) reduced CRA recurrence by 19% compared to placebo (Baron et al. 1999) in subjects with adequate vitamin D levels (Grau et al. 2003).

### 12.4.5 Selenium

Selenium as selenomethionine is an essential micronutrient, which is incorporated into at least 30 selenoproteins after absorption. On the basis of epidemiologic data suggesting that high selenium levels might protect against non-melanoma skin cancer, a randomized trial of selenium supplementation in the form of brewer's yeast was conducted in patients at high risk for this cancer (Clark et al. 1996). The incidence of non-melanoma skin cancer, the primary endpoint of the trial, was not reduced but significant results were reported for several secondary endpoints (Duffield-Lillico et al. 2002). These included reductions in total cancer mortality of 41% and colon cancer incidence of 54%. A phase III trial of selenium supplementation with CRA recurrence as the primary endpoint is now in progress at the Arizona Cancer Center. This study reached its full accrual goal in 2008 and final results are pending completion of the 3-year follow up period for each enrolled participant.

### 12.4.6 Aspirin and Non-Steroidal Anti-Inflammatory Drugs

There is extensive epidemiological, clinical and experimental evidence that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) reduce CRC risk and may reduce mortality from the disease by as much as 40–50% (Thun et al. 2002). NSAIDs inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2. The colorectal and other anti-neoplastic actions of NSAIDs have been attributed largely to inhibition of COX-2 but other actions of these agents that are not related to COX inhibition contribute to their anti-neoplastic activity (Chan et al. 1998; He et al. 1999).

Management of patients with familial adenomatous polyposis (FAP) can consist of colectomy with construction of an ileorectal anastomosis in order to preserve the anal sphincter, thus obviating the need for a permanent ileostomy. In a cohort study, long-term use of sulindac, a non-selective COX inhibitor, reduced CRA burden in the retained rectal segment of most FAP patients following colectomy and ileorectal anastomosis (Cruz-Correa et al. 2002). However, in a prospective RCT, sulindac did not prevent the development of CRAs in subjects with FAP prior to colectomy (Giardiello et al. 2002).

Aspirin has been shown to significantly reduce the risk of developing new CRAs over a period of 1–3 years in RCTs of patients from whom CRCs were recently resected (Sandler

et al. 2003) or CRAs were removed colonoscopically (Baron et al. 2003; Benamouzig et al. 2003). A single daily dose level (325 mg) was used in the study of patients who had undergone CRC resection. Two daily dose levels of aspirin (81 mg, low-dose and 325 mg, standard-dose) were tested in one of the CRA recurrence trials (Baron et al. 2003). Low-dose aspirin, as used for cardiovascular prophylaxis, reduced CRA recurrence by 19% but, rather surprisingly, the recurrence rate was not decreased in the group that received the 325 mg dose.

The effect of aspirin dose and duration of use on CRA risk was clarified in another report from the Nurses' Health Study (Chan et al. 2004). The greatest effect was evident at a weekly total dosage of at least 14 standard-dose tablets, which is much higher than is recommended for cardiovascular prophylaxis. Similar dose-response relationships were found among regular short-term users (5 years or less) and long-term users (more than 5 years). Genetic factors may further modify dose-related effects of aspirin on CRA and CRC risk. As mentioned, a polymorphism of the *ornithine decarboxylase* gene was found to enhance substantially the protective effect of aspirin against CRA recurrence (Martinez et al. 2003).

Potentially life-threatening gastrointestinal (hemorrhage), cerebrovascular (hemorrhagic stroke) and renal complications occur with sufficient frequency to preclude the unrestricted long-term use of standard-dose aspirin therapy for chemoprevention of CRC. Even low-dose aspirin is associated with some risk for hemorrhagic stroke (He et al. 1998) and gastrointestinal hemorrhage (Cryer 2002). The complications of aspirin are attributed largely to inhibition of COX-1 and the chemopreventive activity to inhibition of COX-2. Selective COX-2 inhibitors, the coxibs, were developed in the hope that complications would be minimized. The first two clinically available coxibs were celecoxib and rofecoxib. Although sulindac did not prevent the development of CRAs in subjects with FAP (Giardiello et al. 2002), celecoxib at the higher of two dose levels (400 mg twice daily) significantly reduced polyp burden after 6 months in patients with FAP (Steinbach et al. 2000). Celecoxib and rofecoxib were shown in a nested case-control study using data from a government insurance database to protect against the development of colorectal neoplasia (Rahme et al. 2003). On the basis of the promising results from observational studies, several RCTs of coxibs using the CRA recurrence model were initiated but have yet to be reported. The process of evaluating coxib anti-neoplastic efficacy was recently jolted by an interim report of an excess of 16 myocardial infarctions or strokes per 1,000 patients in a rofecoxib (VIOXX®) CRA recurrence trial (Fitzgerald 2004; Topol 2004). As a result, Merck, the manufacturer, abruptly withdrew VIOXX® from the worldwide market. Although celcecoxib-related cardiovascular toxicity has so far not been reported from CRA recurrence trials of this agent, a definitive statement on the anti-neoplastic efficacy and safety of the coxibs as a class would be premature.

### 12.4.7 Ursodeoxycholic Acid

Deoxycholic acid (DCA) is the predominant fecal secondary bile acid. Combined epidemiological, experimental and clinical data support the hypothesis that fecal DCA concentration is positively associated with CRC risk. Ursodeoxycholic acid (UDCA) is

physiologically present in very low concentrations in human bile and at pharmacological oral doses given long-term can dissolve small, translucent gall stones. UDCA also reduces fecal DCA concentration, providing the rationale for its potential use as a chemopreventive agent for colorectal neoplasia, and is devoid of significant toxicity. The current main clinical indication for chronic UDCA use is treatment of the potentially fatal primary disorders of bile ducts, primary biliary cirrhosis and primary sclerosing cholangitis. UDCA reduced the rate of CRA recurrence in an observational study of primary biliary cirrhosis patients taking UDCA (Serfaty et al. 2003). UC is associated with primary biliary cirrhosis and also, as described, with increased CRC risk. UDCA was strongly associated with decreased prevalence of colonic dysplasia in patients with UC and primary sclerosing cholangitis in an observational study (Tung et al. 2001) and a RCT (Pardi et al. 2003). UDCA (13–15 mg/kg daily) significantly decreased CRC risk in patients with UC and primary sclerosing cholangitis (Pardi et al. 2003). The magnitude of this effect, first apparent approximately 3 years after starting UDCA therapy, continued to increase with lengthening duration of therapy.

In a large RCT, although UDCA (8–10 mg/kg daily for 3 years) did not reduce the rate of CRA recurrence, there was a statistically significant 39% reduction in the recurrence of CRAs with high-grade dysplasia (Alberts et al. 2005). It is possible that the relatively low UDCA dose and short 3-year duration of the trial contributed to the lack of a significant effect on total CRA recurrence.

### 12.4.8 Current Status of CRC Chemoprevention

RCT evidence for macronutrient interventions to reduce risk for colorectal neoplasia is lacking. However, diets that are high in fiber, fruit and vegetable content and low in unsaturated fats confer multiple other well validated preventive health benefits. It remains possible that they reduce CRC risk and there are no significant adverse effects. Micronutrient folate and calcium supplements can safely be taken by people in the general population and have been shown to protect against colorectal neoplasia, besides their other benefits. There are no grounds for recommending pharmacological chemoprevention to the population at average CRC risk and the same applies for those average-risk people who have had one or two non-advanced adenomas removed. The latter group should follow current recommendations for surveillance colonoscopy. People taking low-dose aspirin for cardiovascular prophylaxis may also be protected against colorectal neoplasia.

Standard-dose aspirin chemoprevention should be restricted to people at increased CRC risk, for whom the decision to recommend aspirin chemoprevention should usually be individualized by a specialist. The risks of peptic ulceration, complicated by perforation or hemorrhage, and hemorrhagic stroke must be considered. Screening and surveillance protocols should not be relaxed in subjects at increased risk for CRC who are taking chemopreventive therapy. UDCA continues to show promise for CRC chemoprevention and is remarkably safe. Approaches to chemoprevention are likely to change substantially as the results of RCTs become available.

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# Lung Cancer Prevention

# 13

I. Hakim, L. Garland

Lung cancer continues to exact a huge toll on the health status of Americans and people worldwide. In the United States (US), the number of new lung cancer cases diagnosed per year has reached epidemic proportions. In 2008, an estimated 215,020 new cases of lung cancer were diagnosed, representing 15% of the 1,437,180 new cases of all cancers diagnosed in 2008 (Jemal et al. 2008). While prostate cancer and breast cancer lead in the number of new cancer cases in American men and women, lung cancer remains the leading cause of cancer-related death for both men and women, with an estimated 161,840 of all 565,650 cancer deaths, representing 28.6% of all cancer cases attributable to lung cancer. While once thought to be mainly a man's disease, lung cancer is now represented in a nearly equal fashion between the sexes, with women diagnosed with lung cancer in 2008 representing a full 47% of all new lung cancer cases (Jemal et al. 2008).

## 13.1 The Epidemiology of Lung Cancer

### 13.1.2 Trends in Tobacco Use in the United States

The lung cancer epidemic that has now manifested in the US had its roots in the tremendous increase in smoking prevalence through the 1900s. In the early 1900s, smoking, especially among women, was relatively rare (USDHHS 1980). Over the next 50 years, smoking prevalence increased dramatically, influenced by expanding tobacco marketing initiatives by the tobacco industry. Both male and female smokers were cultivated; in fact, as early as the 1920s the tobacco industry first began its targeting of women utilizing the concept of 'image advertising,' offering lipstick-colored cigarette tips for the woman smoker and

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developing advertising campaigns illustrated by the slogan, "Reach for a Lucky instead of a sweet" to create the association between cigarette use with staying slim, a theme particularly appealing to women (Wallace 1929). There was a significant increase in the numbers of men and women who took up the habit of smoking cigarettes during World War II, with cigarettes being included in government issue ration kits, and image advertising capitalizing on the war effort to promote smoking among women.

### 13.1.3 The Narrowing of the Gender Gap in Smoking Prevalence

While early in the century a large gender gap in smoking prevalence existed, the gap narrowed significantly over the middle and latter parts of the century as a consequence of several trends. First, women's use of cigarettes virtually soared over the mid-1900s in the face of aggressive niche marketing that linked women's smoking to their burgeoning social and political independence, a marketing effort best typified by the Virginia Slims advertising slogan, "You've Come a Long Way, Baby." Second, in the 1950s, the first epidemiologic studies were conducted that definitively linked tobacco exposure and lung cancer (Levin et al. 1950; Doll and Hill 1952). In 1964, the influential report of the Advisory Committee to the Surgeon General cited evidence of the adverse health effects of tobacco use (USDEW 1964). At that time, 51.9% of men and 33.9% of women were smoking (Giovino et al. 1994).

In 1964, the Surgeon General's Report on Smoking and Health became the first national declaration of the association between cigarette smoking as a cause of cancer and other diseases. The publication of this report was followed in 1965 by a congressional act requiring a general health warning on all cigarette packaging regarding the dangers of cigarette smoking. The landmark 1964 Surgeon General's Report on Smoking and Health (USDHHS 1964) provided official evidence that cigarette smoking is a cause of cancer and other serious diseases. The following year, Congress passed the Federal Cigarette Labeling and Advertising Act, requiring health warnings on all cigarette packages: "Caution: Cigarette Smoking May be Hazardous to Your Health."

A wave of aggressive private, state and federal-based tobacco control initiatives followed, promoting smoking cessation and placing restrictions on some venues for tobacco advertisements such as broadcast advertising on televisions, bans on billboard advertising, and restrictions on sales and advertising to children and adolescents. For men, the latter part of the century saw a decline in smoking prevalence; for women, smoking prevalence continued to increase. Thus, at the very end of the twentieth century, the gender gap had narrowed to only around 5%, with 22% of women aged 18 or older in the US smoking cigarettes, compared to 26.4% of US men. Figure 1 illustrates the trend in all adult cigarette smoking over the twentieth century in relation to public health milestones.

While the decline in overall cigarette use in the US over the latter part of the twentieth century is considered to have been one of great public health achievements of that century, there continues to be about 48 million adult smokers, half of whom will die of smoking-related disease. Economically, the burden of tobacco use continues to exact a staggering $50 billion in medical expenditures and another $50 billion in indirect costs such as lost wages (1999). To continue to aggressively address the tremendous public health burden derived from tobacco use, the Department of Health and Human Services issued a national

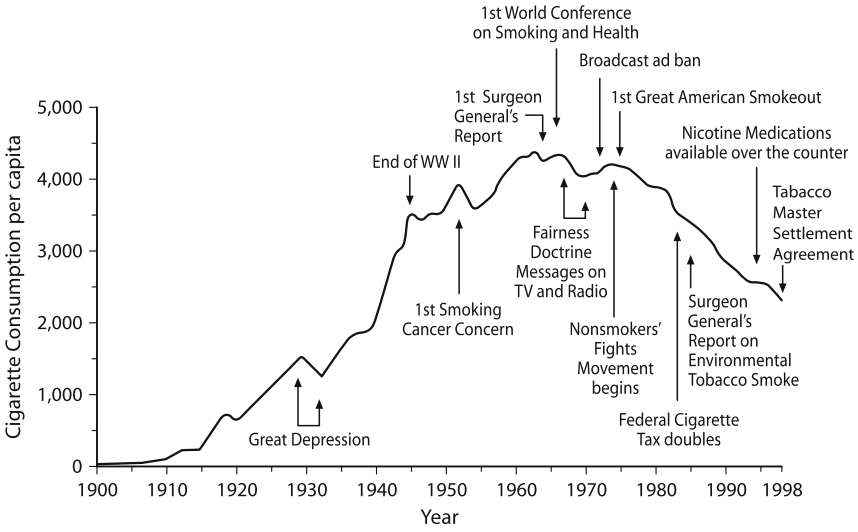


**Fig. 1** Annual adult per capita cigarette consumption and major smoking and health events – Unites States, 1990–1998 – Source of data: US Department of Agriculture, 2000 Surgeon General's Report

health objective for the United States for the year 2010 to reduce the prevalence of cigarette smoking among adults to less than 12% (USDDHS 2000).

### 13.1.4 Demographic Variables and Tobacco Use

Tobacco use varies by a number of variables apart from gender; these include age, education, socioeconomic status and ethnicity/race. A Centers for Disease Control (CDC) analysis of self-reported data from the 2000 National Health Interview Survey (NHIS) sample showed smoking prevalence was highest among adults 18–44 years of age and lowest among adults aged greater than 65 years of age. Cigarette use varied inversely with level of education, with a prevalence of 47.2% in adults with a general education degree as compared to a prevalence of 8.4% among adults with a master's, professional or doctoral degree. Persons living below the poverty level had higher prevalence of smoking than persons at or above the poverty level (31.7% versus 22.9%, respectively) (2002).

Cigarette use varies widely by ethnic/racial groups. Factors that have been implicated in the complex interactions that influence tobacco use by ethnic group include socioeconomic status, cultural factors and norms, acculturation, biologic factors, impact of advertising targeted by ethnicity, price of tobacco products, and variation in the ability of communities to implement effective tobacco-control initiatives (2004). Smoking prevalence among five defined ethnic/racial groups in the US varies widely (Table 13.1), with Asians (14.4%) and Hispanics (18.6%) having the lowest prevalence of cigarette use while American Indians/Alaska Natives having the highest prevalence (36.0%) (2002). The need to tailor tobacco

**Table 13.1** Percentage of persons aged ≥18 years who were current smokers[a], by selected characteristics – National Health Interview Survey, United States, 2000, reprinted with permission from Morbidity and Mortality Weekly Report (2002)

| | Men (n=13,986) | | Women (n=18,388) | | Total (n=32,374) | |
|---|---|---|---|---|---|---|
| **Characteristic** | % | (95% CI[b]) | % | (95% CI) | % | (95% CI) |
| **Race/Ethnicity[c]** | | | | | | |
| White, non- Hispanic | 25.9 | (±1.0) | 22.4 | (±0.8) | **24.1** | **(±0.7)** |
| Black, non-Hispanic | 26.1 | (±2.5) | 20.9 | (±1.7) | **23.2** | **(±1.5)** |
| Hispanic | 24.0 | (±2.1) | 13.3 | (±1.6) | **18.6** | **(±1.3)** |
| American Indian/ Alaska Native[d] | 29.1 | (±11.0) | 42.5 | (±11.0) | **36.0** | **(±8.0)** |
| Asian[e] | 21.0 | (±4.6) | 7.6 | (±2.8) | **14.4** | **(±2.8)** |
| **Education[f]** | | | | | | |
| 0–12 (no diploma) | 33.2 | (±2.2) | 23.6 | (±1.7) | **28.2** | **(±1.4)** |
| ≤8 | 26.1 | (±3.1) | 14.2 | (±2.2) | **20.0** | **(±1.9)** |
| 9–11 | 37.6 | (±3.5) | 30.8 | (±2.7) | **33.9** | **(±2.2)** |
| 12 | 40.1 | (±6.8) | 25.3 | (±5.1) | **32.7** | **(±4.4)** |
| GED[g] diploma | 50.1 | (±6.2) | 44.3 | (±5.7) | **47.2** | **(±4.3)** |
| 12 (diploma) | 31.7 | (±1.9) | 23.5 | (±1.4) | **27.2** | **(±1.2)** |
| Associate degree | 21.9 | (±2.8) | 20.4 | (±2.4) | **21.1** | **(±1.8)** |
| Some college | 25.8 | (±2.1) | 21.6 | (±1.7) | **23.5** | **(±1.3)** |
| Undergraduate degree | 14.2 | (±1.7) | 12.4 | (±1.5) | **13.2** | **(±1.1)** |
| Graduate degree | 9.1 | (±1.8) | 7.5 | (±1.6) | **8.4** | **(±1.2)** |
| **Age group (years)** | | | | | | |
| 18–24 | 28.5 | (±2.7) | 25.1 | (±2.4) | **26.8** | **(±1.8)** |
| 25–44 | 29.7 | (±1.4) | 24.5 | (±1.1) | **27.0** | **(±0.9)** |
| 45–64 | 26.4 | (±1.5) | 21.6 | (±1.3) | **24.0** | **(±1.0)** |
| 65 | 10.2 | (±1.3) | 9.3 | (±1.0) | **9.7** | **(±0.8)** |
| **Poverty status[h]** | | | | | | |
| At or above | 25.4 | (±1.0) | 20.4 | (±0.9) | **22.9** | **(±0.7)** |
| Below | 35.3 | (±3.2) | 29.1 | (±2.3) | **31.7** | **(±1.9)** |
| Unknown | 23.6 | (±1.8) | 19.5 | (±1.4) | **21.4** | **(±1.1)** |
| **Total** | 25.7 | (±0.8) | 21.0 | (±0.7) | **23.3** | **(±0.5)** |

[a] Smoked ≥100 cigarettes during their lifetime and reported at the time of interview smoking every-day or some days. Excludes 301 respondents for whom smoking status was unknown.
[b] Confidence interval.
[c] Excludes 287 respondents of unknown, multiple, and other racial/ethnic categories.
[d] Wide variances among estimates reflect limited sample sizes.
[e] Does not include Native Hawaiians and Other Pacific Islanders.
[f] Persons aged ≥25 years. Excludes 305 persons with unknown years of education.
[g] General Education Development certificate (US high school equivalency diploma)
[h] The 1999 poverty thresholds from the Bureau of the Census were used in these calculations

control interventions by cultural demographics is underscored by a further analysis of the NHIS data showing that even within each of the four primary racial/ethnic minority populations (non-Hispanic blacks, American Indians/Alaska Natives, Asians/Pacific Islanders, and Hispanics), cigarette use by youth and adults varies widely (2004).

## 13.2 Etiology of Lung Carcinogenesis

### 13.2.1 Oxidative Damage

Oxidative damage is implicated in several chronic diseases including cancer and chronic inflammation. Oxidative reactions have been implicated as important modulators of human health and can play a role in both disease prevention and disease development. Small-cell carcinoma of the lung (SCLC) is a highly malignant systemic disease characterized by rapid and widespread dissemination of tumor cells at the time of diagnosis (Erhola et al. 1997). Decreased plasma peroxyl radical trapping capacity was reported in SCLC patients (Erhola et al. 1997). The most enhanced lipid peroxidation in tumor tissue was noted in specimens of adenocarcinoma and SCLC tissue, in case of which 'early' dissemination and fast growth are common features (Zieba et al. 2000). Thus it appears that lung cancer of these histologic types is associated with an increased oxidative stress that is most likely due to the systemic nature of the disease (Erhola et al. 1997). Oxidative damage to the DNA of key cellular genes is a fundamental event leading to malignancy (Ames et al. 1995), whereas cellular generation of oxidants is important in the control of infectious agents (Peterhans 1997; Akaike et al. 1998) and eliminating newly developed tumors (Farias-Eisner et al. 1994; Filep et al. 1996; Yamashita et al. 1997). Cellular-generated small molecules, such as nitrogen oxides and oxygen radicals, have the potential to cause significant genetic and cellular damage (Ames and Shigenaga 1992; Keefer and Wink 1996), yet they are also key cellular signaling molecules (Lane and Gross 1999), which may either protect against or enhance the development of malignancy.

The sources of increased oxidative stress derive from the increased burden of oxidants present in cigarette smoke, or from the increased amounts of reactive oxygen species released from leukocytes into the airspaces and blood (MacNee 2001). Oxidative processes have fundamental roles in inflammation through redox-sensitive transcription factors, such as NF-κB and AP-1, that regulate the genes for proinflammatory mediators and through protective mechanisms, such as antioxidant gene expression. In addition to the oxidative stress produced by cigarette smoking, dietary deficiency in antioxidants is shown to be related to the development of airflow limitation (MacNee 2001). Hence dietary supplementation may be a beneficial therapeutic intervention in this condition.

A common oxidative damage to DNA is the highly mutagenic 7,8-dihydro-8-oxoguanine adduct, which can be repaired by 8-oxoguanine glycosylase I (OGG1). The human homologue of the yeast OGG1 gene, hOGG1, has been cloned, and its genetic structure has been determined. Several polymorphisms in the hOGG1 gene were detected in humans. The distributions of this polymorphism varies for different populations, and among the different polymorphisms, the Ser-Cys polymorphism at codon 326 has been suggested to reduce the activity of the enzyme. Because many environmental carcinogens produce 8-hydroxyguanine residue and mismatching to this modified base potentially causes oncogenic mutations, the capacity to repair these lesions can be involved in cancer susceptibility in human beings. Published data suggest that the presence of two hOGG1 326Cys alleles confers a twofold increased risk of lung cancer (Le Marchand et al. 2002).

Although the specific mechanisms by which oxidative stress contributes to the development of carcinogenesis are largely unknown, oxidative DNA damage is thought to play a role in the development of carcinogenesis via at least two different mechanisms. In the first mechanism, genetic alterations induced by oxidants, such as mutations and chromosomal rearrangements, can play a role in the initiation and malignant conversion stages of carcinogenesis (Guyton and Kensler 1993). Most oxidative DNA damage results in a wide range of chromosomal abnormalities, causing a blockage of DNA replication and wide cytotoxicity (Bohr et al. 1995). Mutations can occur through misrepair or due to incorrect replication past a damaged site, while chromosomal rearrangements can result from strand breakage misrepair (Halliwell and Aruoma 1991; Bohr et al. 1995). These genetic alterations can result in permanent DNA damage and a population of initiated cells that must escape repair processes, overcoming cytotoxicity in order to be carried on to the progeny. The initiation potential of oxidants may be due to their ability to induce DNA base changes in certain oncogenes and tumor suppressor genes, contributing to carcinogenesis (Jackson 1994). Hydroxy radicals have been demonstrated to activate certain oncogenes, such as K-ras and C-Raf-1, respectively, through the induction of DNA point mutations in GC base pairs and N-terminal deletions in these genes (Jackson 1994). Base point mutations in CpG dinucleotides are also frequently found in certain tumor suppressor genes, such as p53 and retinoblastoma, leading to their inactivation (Nigro et al. 1989; Yandell et al. 1989). Furthermore, hydroxy radical exposure of cells that contain mutant or absent p53 resulted in a failure to arrest in G1, reducing their capacity to repair damaged DNA (Jackson 1994). This increase in replication errors can compromise DNA fidelity, predisposing initiated cells to undergo additional oncogene activation and tumor suppressor gene inactivation, ultimately contributing to malignancy (Jackson 1994). Oxidant-induced cytotoxicity may also contribute to the initiation of carcinogenesis by depleting the normal cell population, promoting the clonal expansion of more resistant initiated cells, thus increasing the probability of mutation.

Among various markers of DNA damage, 8-hydroxydeoxyguanosine (8-OHdG), an oxidative adduct form of deoxyguanosine, is considered to be one of the most sensitive (Floyd et al. 1986). 8-OHdG is induced by several carcinogens and tumor promoters (Floyd 1990; Takeuchi et al. 1994; Shen et al. 1995) and causes mutation both in vitro and in vivo (Wood et al. 1990; Cheng et al. 1992). 8-OHdG occurs specifically in DNA and appears to be a reasonable marker for oxidative DNA damage (assuming a steady state) because the rate of output of 8-OHdG by repair should balance the rate of input of damage. Toyokuni and co-workers (Toyokuni et al. 1995) reported that human carcinoma cells (breast, lung, liver, kidney, brain, stomach, ovary) have a higher content of 8-OHdG than adjacent non-tumorous tissues. Moreover, investigators have reported a high concentration of 8-OHdG in lung cancer tissues (Inoue et al. 1998). They hypothesized that the tumor cells themselves produce ROS spontaneously, which results in an increase of 8-OHdG in DNA. 8-OHdG levels in DNA of leukocytes and the central part of the lung were significantly associated with the number of cigarette smoked (Kasai 1998). An increased level of 8-OHdG was found in peripheral part of the lung from lung cancer patients when compared to non-cancer controls (Inoue et al. 1998). Lung cancer patients showed higher levels of urinary 8-OHdG/creatinine than the controls. Furthermore, patients with complete or partial response to chemotherapy showed a significant decrease in urinary 8-OHdG/creatinine while patients with no change or progressive disease showed an increase. Nevertheless,

8-OHdG in blood DNA rather than urinary 8-OHdG might be a better marker of oxidative damage because the latter might reflect the repair process.

A group of prostaglandin (PG)-like compounds, known as isoprostanes, has been discovered in the late twentieth century. 8-F2 isoprostanes (8-epi-PGF2) have been measured as indices of lipid peroxidation in body fluids such as urine, blood, bile (Leo et al. 1997; Pratico et al. 1998a) pericardial (Mallat et al. 1998) and cerebrospinal fluid (Pratico et al. 1998a,b; Montine et al. 1999), and lung condensate (Montuschi et al. 1998). Isoprostanes are formed from arachidonic acid in vivo, not by involvement of oxidizing enzymes, such as cyclooxygenase, but by free radical-catalyzed peroxidation. Free F2-isoprostanes are released from the esterified stores on the cell surface by the action of phospholipases (Ohashi and Yoshikawa 2000). Therefore, the amount of isoprostanes should reflect the levels of oxidant stress and free radicals in vivo (Morrow et al. 1994; Awad et al. 1996).

## 13.3 Cell Proliferation and Lung Carcinogenesis

Lung cancer, like many other epithelial malignancies, is thought to be the outcome of genetic and epigenetic changes that result in a constellation of phenotypic abnormalities in bronchial epithelium. These include morphologic epithelial dysplasia, angiogenesis, increased proliferative rate, and changes in expression of cell surface proteins, particularly overexpression of epidermal growth factor receptor (EGFR) family proteins. EGFR overexpression is pronounced in virtually all squamous carcinomas and is also found in 65% of large cell and adenocarcinomas. Overexpression of EGFR is one of the earliest and most consistent abnormalities in bronchial epithelium of high-risk smokers. It is present at the stage of basal cell hyperplasia and persists through squamous metaplasia, dysplasia, and carcinoma in situ (Khuri et al. 2001).

The expression level of the proliferating cell nuclear antigen (PCNA) was associated with the histological grade of the bronchial biopsy site. Intervention with 13-cic-retinoic acid augmented the decreased proliferation status and decreased metaplasia index associated with discontinuation of smoking but had little impact on the proliferation status of the bronchial epithelium in those who continued to smoke (Khuri et al. 2001). The level of PCNA expression in the bronchial epithelium correlated with the degree of EGFR expression, which is also found to be increased in metaplastic lesions (Hommura et al. 2000). Another proliferation marker, Ki-67, has been shown to be increased in lung tumors and to provide some prognostic information (Nguyen et al. 2000; Hittleman 2002). In patients who had stopped smoking, the Ki-67 labeling index dropped significantly within a year and continued to drop thereafter. However, abnormal levels of Ki-67 labeling are detectable for more than 20 years after smoking cessation (Lee et al. 1998). The Activator Protein-1 (AP-1) complex is a dimeric transcription factor composed of fos and jun proteins that regulates cellular growth and differentiation. Lee and co-workers (Lee et al. 1998) demonstrated a reduction in basal AP-1 transcriptional activity was associated with the malignant transformation of human bronchial epithelial cells that was, in part, a consequence of decreased c-fos expression.

## 13.4 Apoptosis and Lung Carcinogenesis

Use of biomarkers to predict induction of apoptosis allows identification of biological signs that may indicate increased risk for disease. In cells undergoing apoptosis, the release of cytochrome c from the mitochondria to the cytoplasm and the activation of caspase-3, a key enzyme in the execution stage of apoptotic pathway, have been studied as biomarkers of apoptosis (Koomagi and Volm 2000). A significant correlation was observed between the expression of caspase-3, survival and metastasis in 135 non-small cell lung carcinomas. Caspase-3 expression correlated with a lower incidence of lymph node involvement ($p = 0.0007$). The median survival was longer for patients with caspase-3-positive carcinomas than for those with caspase-3-negative tumors (Chen et al. 1999).

Apoptosis is a highly programmed process regulated by many genes, including Bcl-2 family genes (Wang et al. 2000). The Bcl-2 proto-oncogene, an indirect measure of apoptosis, is known to promote cell survival and to act as a negative regulator of the biological cascade that leads to apoptosis and to provide a growth advantage eventually leading to neoplastic transformation (Lee et al. 1998). The mRNA expressions of the Bcl-2 gene were studied in a series of 137 pulmonary tissues collected at various sites and with different properties. According to the observations on benign lesions, non-cancer tissues distant from tumor, para-tumor tissues and cancer tissues, there was a trend toward increased Bcl-2 mRNA expression. Among them, Bcl-2 mRNA expression in lung cancer tissues was significantly increased as compared to benign lesions and tissues distant from tumor ($p < 0.01$) (Yang et al. 1998). Expression of the Bcl-2 protein has been reported for a variety of tumors, including the lung. The overexpression of Bcl-2 is thought to be early event in carcinogenesis, allowing cells with DNA damage to escape the normal mechanisms of apoptotic cell death. Conversely, the loss of Bcl-2 expression may be relatively late in the pathogenesis of lung cancer (Wang et al. 2000).

## 13.5 Genetic Factors in Carcinogen Metabolism

Cigarette smoke contains numerous compounds that generate reactive oxygen species (ROS) that can damage DNA directly or indirectly via inflammatory processes (Frenkel et al. 1988; Wei et al. 1993; Hecht 1999). Oxidants, either present in cigarette smoke and/or formed in the lungs of smokers, may trigger oxidative damage to DNA and cellular components, contributing to carcinogenesis. Free radical attack upon DNA generates a multiplicity of DNA damage, including modified bases. Some of these modifications have considerable potential to damage the integrity of the genome.

DNA damage was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential. ROS are highly biologically active chemicals. They may interact with DNA and damage its structure. Because the human population is biologically diverse and genetically heterogeneous, it is not surprising that differences in

susceptibility to disease among individuals with or without exposure to environmental agents exist. Individuals vary greatly in their susceptibility to disease. This is true of adults and children. The etiologies of many diseases of childhood are due to a combination of factors, including genetic susceptibility and environmental exposures during vulnerable periods of development. Genes regulate cellular growth and development, DNA replication and repair, the metabolism of endogenous agents in the body, and the metabolism and excretion of exogenous agents that the body comes in contact with in the environment. This regulation varies over the life span, contributing to the cellular consequences of the environmental exposures.

## 13.6 DNA Hypermethylation and Lung Carcinogenesis

Methylation is the main epigenetic modification in humans; changes in methylation patterns play an important role in tumorigenesis. Aberrant promoter methylation has been described for several genes in various malignant diseases including lung cancer (Esteller et al. 1998; Esteller et al. 1999a; Esteller et al. 2000). In a large study of primary resected non-small cell lung cancer (NSCLC), a high frequency of methylation was observed, demonstrating that methylation may be the most common mechanism to inactivate cancer-related protection genes in NSCLC (Zochbauer-Muller et al. 2001).

The tumor suppressor gene (p16), DNA repair gene (MGMT), and genes related to metastasis and invasion (DAP-K and TIMP3) are well characterized. Each possess a CpG island in the 5' region which is unmethylated in normal tissues, as expected for a typical CpG island (Esteller et al. 2001). Methylation of p16, MGMT, DAP-K and TIMP3 has been described in lung cancer cell lines and a small number of primary lung tumors (Esteller et al. 1999a). Furthermore, when these CpG islands were hypermethylated in cancer cells, expression of the corresponding gene was silenced. The silencing was partially reversed by demethylation of the promoter region (Esteller et al. 1999a). Thus, chemopreventive agents that have the ability to demethylate these genes may be able to restore their function and help slow or prevent carcinogenesis.

Belinsky and co-workers (Belinsky et al. 1998) were the first to demonstrate that inactivation of the p16 tumor suppressor gene by aberrant methylation is an early and likely critical event in the development of NSCLC. Palmisano et al. (Palmisano et al. 2000) corroborated Belinsky's work, reporting that p16 hypermethylation was detected in 60–80% of squamous cell carcinoma (SCC), and 30–45% of adenocarcinomas. Several other studies have shown that inactivation of the p16 tumor gene is common in lung cancer (Belinsky et al. 1998; Kersting et al. 2000) and that methylation of the p16 gene is clearly associated with loss of gene transcription in lung tumors (Belinsky et al. 1998). P16 methylation has also been observed in the precursor lesions of SCC, including basal cell hyperplasia, squamous metaplasia and carcinoma in situ of the lung. The frequency of p16 methylation increased from the lowest to highest-grade precursor lesions to SCC (Kersting et al. 2000). The high frequency of p16 methylation in alveolar hyperplasias and adenomas – precursor lesions with an extremely high conversion rate to adenocarcinomas in NNK-treated rats – indicates that p16 hypermethylation is an early molecular event in lung carcinogenesis and thus a sound candidate biomarker for lung chemoprevention trials (Belinsky et al. 1998).

The DNA repair protein, $O^6$-methylguanine DNA methyltransferase (MGMT), is a major determinant of susceptibility to methylating carcinogens and of tumor resistance to chloroethylating drugs (Danam et al. 1999). MGMT protein expression is decreased in some human tumors, including lung, with respect to their normal tissue counterparts. Loss of expression is rarely due to deletion, mutation or rearrangement of the MGMT gene. However, the methylation of discrete regions of the CpG island of MGMT is associated with the silencing of the gene in cell lines (Esteller et al. 1999a). Aberrant methylation of MGMT was detected in 21–29% of NSCLCs (Esteller et al. 1999a; Esteller et al. 2001). Palmisano et al. (Palmisano et al. 2000) detected MGMT methylation in epithelial cells shed from the airways in persons at risk for lung cancer, and reported a frequency of 16% in samples investigated. In contrast, methylation of MGMT has not been observed in normal lung tissue (Esteller et al. 1999a).

Death-associated protein (DAP) kinase, also known as DAP-2, is a novel serine/threonine kinase required for interferon gamma-induced apoptotic cell death (Tang et al. 2000) that may function as a metastasis suppressor. Expression of DAP kinase was repressed in human cancers by hypermethylation in the promoter CpG region (Esteller et al. 1999b; Tang et al. 2000). Esteller (Esteller et al. 1999b) studied primary NSCLC samples from 22 patients and found that DAP kinase was hypermethylated in five (23%) of the 22 tumors. In 135 lung tumors, 44% of the tumors were hypermethylated at the CpG sites of the DAP kinase gene; DAP kinase methylation was negatively associated with the expression of DAP kinase in lung cancer cell lines and demethylation restored DAP kinase gene expression (Tang et al. 2000).

DNA repair plays a critical role in protecting the genome of the cell from insults of cancer-causing agents, such as those found in tobacco smoke. Reduced DNA repair capacity, therefore, can increase the susceptibility to smoking-related cancers. Recently, three coding polymorphisms in X-ray cross-complementing group 1 (XRCC1) DNA repair gene have been identified, and it is possible that these polymorphisms may affect DNA repair capacity and thus modulate cancer susceptibility. Polymorphisms of XRCC1 appear to influence risk of lung cancer and may modify risk attributable to environmental exposures. A recent published study suggests that XRCC1 codon 399 polymorphism may be an important genetic determinant of SCC of the lung in persons with lower amounts of cigarette use (Park et al. 2002).

## 13.7 Risk Factors for Lung Cancer

### 13.7.1 Tobacco Products

Cigarette smoking, the primary risk factor for lung cancer, accounts for approximately 90% of cases in men and 70% of cases in women (Shopland 1995; Jemal et al. 2001). A dose-response relationship between daily tobacco smoking and risk of death from lung cancer has been established in prospective analyses, with the relative risk of lung cancer mortality ranging from 4.6 to 7.8 in users of less than ten cigarettes per day, compared

to never smokers. This relative risk increases to more than 20 in individuals who smoke 25–40 or more cigarettes per day (Hammond 1966; Rogot and Murray 1980).

Cigarette smoking may result in chronic bronchitis, chronic obstructive pulmonary disease, and/or lung cancer. In the early 1960s, Passey (Passey 1962) hypothesized that it was the irritating properties of tobacco smoke, resulting in chronic bronchitis and inflammatory destruction of lung tissue, that was of pathogenic significance in the causal pathway of lung cancer, rather than any direct action by volatile and particulate carcinogens in tobacco smoke. The experiments of Kuschner (Kuschner 1968), however, suggested an alternative explanation; namely, that bronchial and bronchiolar inflammation, accompanied by reactive proliferation, squamous metaplasia, and dysplasia in basal epithelial cells, provided a cocarcinogenic mechanism for neoplastic cell transformation upon exposure to polycyclic aromatic hydrocarbons.

Currently, 40–50 million Americans are former smokers (Resnicow et al. 1991). The risk of former smokers developing lung cancer actually increases during the first 3–5 years after smoking cessation; many smokers stop because they are symptomatic and may already have the disease (Hammond 1966). Eventually and gradually, over at least a decade or more, the risk of lung cancer for these individuals will come to approach that of never smokers (Halpern et al. 1993). In several studies, the risk of lung cancer in former smokers had not reached that of lifetime never smokers, even after 20 years since cessation (Khuri et al. 1995; Burns 2000; Ebbert et al. 2003).

Current and former smokers older than 40 years with a smoking history of 20 pack-years or more and airflow obstruction, defined as a forced expiratory volume in one second/forced vital capacity (FEV1/FVC) of 70% or less and a FEV1 lower than 70%, are at high risk of developing lung cancer (Kennedy et al. 1996). Patients with chronic obstructive pulmonary disease (COPD) have a four to sixfold increased risk of lung cancer independent of their smoking history. The chronic inflammation associated with COPD appears to enhance lung cancer risk. At least ten cohort studies have reported that COPD is an independent predictor of lung cancer risk (Tenkanen et al. 1987; Tockman et al. 1987).

Reactive oxygen species (ROS) play an important role in toxicity of environmental chemicals. During passive smoking, the body is attacked by an excess of free radicals inducing oxidative stress. In non-smokers, even a short period of passive smoking breaks down serum antioxidant defense and accelerates lipid peroxidation (Zhang et al. 2001). Tobacco smoke contains many carcinogens that exert their biological effects through interaction of reactive intermediates with DNA to form DNA adducts. The same electrophilic species also react with cellular proteins. The effects of smoking are evident by the detection of elevated levels of carcinogen-DNA adducts in many human tissues and of carcinogen-protein adducts in blood. Components of tobacco smoke also induce oxidative DNA damage (Phillips 2002). Exposure to environmental tobacco smoke resulted in a statistically significant increase of 63% of the oxidative DNA mutagen, 8-OHdG, in the blood of exposed subjects. This oxidative DNA damage has been linked to an increased risk of developing several degenerative chronic diseases, including coronary heart disease and cancer (Howard et al. 1998). Significant effects on oxygen free radical production were found for gender and ethnicity, with men having greater values than women ($p < 0.001$) and white subjects having greater values than black subjects ($p = 0.025$).

Environmental tobacco smoke (ETS) constitutes both a residential and an occupational exposure. Animal data show that ROS introduced by passive smoking may contribute to

K-ras activation as an initiator of a tumor model, possibly through the oxygen-induced DNA damage, and may also contribute to an initial activation and the subsequent down-regulation of protein kinase as a promoter (Maehira et al. 2000). Rats exposed to sidestream cigarette smoke, the major component of ETS, showed significant increases in the accumulation of 8-OHdG in lung DNA (Maehira et al. 2000; Izzotti et al. 2001). Similarly, exposure to sidestream cigarette smoke significantly increased oxidative stress in mouse heart, liver, and lung tissues. In all three tissues, ETS increased the presence of 8-OHdG above the control levels (Howard et al. 1998). The assessment of pathological effects produced by ETS in humans is controversial in epidemiological studies. However, based on a collection of studies, there is an association between exposure to ETS and lung cancer, with a relative risk around 1.2 (Boffetta and Nyberg 2003).

### 13.7.2 Environmental Exposures

Occupational exposures have been estimated to account for up to 20% of all lung cancer diagnoses. Many studies that comprise the body of literature on occupational exposure and lung cancer risk have been based on exposures in male smokers, thus introducing confounders in the analysis of pure exposure risk. Little data is available on the effect of occupational exposures in women and non-smokers. Strong evidence exists for asbestos, ETS, radon prodigy and arsenic as occupational carcinogens in non-smokers (Neuberger and Field 2003). Nonetheless, the International Agency for Research on Cancer (IARC) maintains a much longer list of workplace-related carcinogens, including both chemical and physical agents, implicated in the risk of lung cancer.

Because women have only recently been assimilated into many occupational environments formerly reserved for men, the role of occupational exposures has been estimated to be lower than that for men, around 5%. A more current analysis of occupational risk of lung cancer for women is needed so that a more accurate risk assessment for women in the workplace can be generated.

Worldwide, an increased risk of lung cancer independent of tobacco exposure has been documented for exposure to environmental carcinogens, a term that includes both outdoor and indoor air pollutants as well as contaminants of soil and drinking water. Persons are exposed to environmental carcinogens from both natural and man-made exposures, and exposures occur in both residential and occupational settings. While there appear overall to be relatively small relative risks of cancer following environmental exposure, the impact on worldwide health is great, given a high prevalence of exposures to these carcinogens.

The naturally-occurring radioactive gas radon and its radioactive progeny are sources of exposure to inhaled radioactive substances. Radon exposure occurs in occupational settings such as uranium and tin mines, and in homes built on radon-containing soil. Studies have consistently shown an excess of lung cancer risk in radon-exposed populations. Studies of radon-exposed underground miners have predicted that residential radon would be an important cause of non-tobacco related lung cancer; in fact, residential exposure to radioactive radon and its decay products are estimated to account for 10–12% of all lung cancer deaths in the US (Lubin and Steindorf 1995).

Radon mitigation programs for homes and improved workplace measures to mitigate exposure to radon and other radioactive elements will likely impact favorably on lung cancer risk.

A high incidence of lung cancer has been reported in some Asian women who have a traditionally low prevalence of cigarette smoking. This elevated risk of lung cancer has been related to indoor pollution from cooking and heating sources with chronic exposure to non-vented, potentially mutagenic cooking oil fumes and the carcinogenic metabolic products of heterocyclic amines aerosolized during the cooking of meat at high temperatures (He et al. 1991; Seow et al. 2001).

Outdoor air pollution is composed of complex mixtures of chemical compounds, radionuclides, gas and particulate combustion products and fibers, a number of which are known carcinogens. Major sources of air pollution include industrial and automobile-related fossil fuel combustion, diesel exhaust, power plants and residential sources of emissions. Air pollution as a contributor to the risk of lung cancer has been supported by occupational studies of workers exposed to fossil fuel combustion products. After adjusting for smoking history, exposed workers had a twofold risk of lung cancer relative to non-exposed workers (Doll et al. 1972). Other studies have looked at lung cancer occurrence in populations with differential exposure to air pollutants (e.g., rural versus urban). Other studies have measured tissue biomarkers of exposure to respiratory carcinogens such as levels of benzo(a)pyrene, carcinogenic DNA adducts, chromosomal abnormalities and other measures of genetic damage in relation to exposure to air pollutants. Epidemiologic studies of lung cancer risk related to air pollution must account for a number of variables that are often difficult to quantify, such as concentration of pollutant carcinogens, length of exposure, geographic variables, genetic variables and individual tobacco smoke-related exposure within the study population. Nevertheless, there is a large body of data that supports a role for air pollution contributing independently to the risk of lung cancer, although estimates of lung cancer attributed to air pollutants range from less than 1% (Doll 1978) to 12% (Karch and Schneiderman 1981). The causal relationship between air pollution and lung cancer risk remains of great public health import, given the migration of populations from rural into more urban settings, and the increasing expanding populations worldwide residing in highly polluted cities in many developing parts of the world (Cohen 2000).

### 13.7.3 Family History

There appears to be a small contribution to an individual's risk of developing lung cancer from family history, suggesting a role for genetic susceptibility that is independent of tobacco exposure. A number of studies have shown an increased risk of cancers in relatives of persons with lung cancer. A landmark study reported in 1963 showed suggestive evidence of familial aggregation of lung cancer specifically, with an excess of lung cancer mortality reported in relatives of 270 lung cancer probands (Tokuhata and Lilienfeld 1963). A number of more recent studies have also found an increase in both lung cancer and total cancers in the first-degree relatives of persons with lung cancer; these familial

aggregations of cancer risk have been seen in both smoking *and non-smoking* persons with lung cancer. In an investigation of family cancer history as a risk factor for lung cancer in non-smoking men and women, a population-based case-control study showed an excess of certain cancers, especially lung, aerodigestive tract and female breast cancer in first-degree relatives of non-smoking cases (Mayne et al. 1999).

Lung cancer risk in families is influenced by familial aggregation of smoking habits; this variable is an important potential confounder of studies investigating pure genetic risk related to lung cancer. A recently reported large case-control study of persons with lung cancer and their first degree relatives investigated tobacco exposure-specific familial risk of lung and other smoking-related cancers. Eight hundred and six persons with lung cancer and 663 controls matched to the cases on age (within 5 years), sex, ethnicity, and smoking history addressed whether there was an excess of cancer in relatives of persons with lung cancer (Etzel et al. 2003). Cancer family history data were available for 6,430 first-degree relatives of the cases and 4,936 first-degree relatives of the controls. Adjustment was made for smoking history and age of lung cancer cases and their relatives. In first degree relatives of lung cancer cases, there was an significantly increased risk of smoking-related cancers (defined as cancers of the lung, bladder, head and neck, kidney, and pancreas) and lung cancer specifically. Relative risks were 1.28 for smoking-related cancer and 1.33 for lung cancer. Additionally, there was a sevenfold increased risk of breast cancer among daughters of lung cancer cases (Etzel et al. 2003). These data suggest that genetic factors modulate lung cancer risk independent of tobacco exposure, and that a better understanding of these factors may influence the way in which the health of families of lung cancer patients are monitored.

### 13.7.4 Genetic Susceptibility

Although smoking is the major risk factor for lung cancer, other factors, such as nutrition or genetic predisposition, may be involved. Genetic susceptibility to environmental carcinogens is thought to be attributable to genetic polymorphisms in metabolism enzymes, which have been found to substantially alter the activation and elimination of carcinogens (Smith et al. 1994).

Glutathione S-transferases (GSTs) constitute a complex multi-gene family that, in most instances, deactivates carcinogens, environmental pollutants, drugs, and a broad spectrum of other xenobiotics through conjugation with glutathione (Hayes and Pulford 1995). Therefore, GST induction may improve detoxification and excretion of potentially harmful compounds. Polymorphic variants in GSTs, l (GSTM1), h (GSTT1), and p (GSTP) have been studied extensively in relation to cancer etiology. Complete gene deletions in GSTM1 and GSTT1 and single nucleotide polymorphism in GSTP may result in a significant change in the function of the enzymes.

Both GSTM1 and GSTT1 are polymorphic, and the null alleles of these genes have deletions of the entire protein-coding region (Seidegard et al. 1988; Pemble et al. 1994). The GSTM1-null and GSTT1-null alleles are transmitted as autosomal recessive, with the phenotypic absence of the isozymes resulting from inheritance of a null allele from both

parents. The prevalence of GSTM1-null and GSTT1-null genotypes differ markedly across ethnic and racial groups (GSTM1, 30–60%; GSTT1, 9–64%) (Bell et al. 1993; Katoh et al. 1996). GSTM1-null and GSTT1-null genotypes have been associated with increased risk of cancer in a number of studies, and it is hypothesized that individuals with putative high-risk genotypes suffer higher levels of carcinogen-induced genotoxic damage (Bell et al. 1993; Rebbeck 1997).

Among the several classes of GSTs, GSTl enzyme activity has been found to vary substantially between individuals because of an inherited deletion of the GSTM1 gene. GSTM1 is involved in the detoxification of tobacco smoke carcinogens including the polyaromatic hydrocarbons (PAHs) such as benzo(a)pyrene (Ketterer et al. 1992). Up to 50% of Whites have no GSTM1 enzyme because of the homozygous deletion of the gene (Seidegard et al. 1988), referred to as the GSTM1-null genotype. Individuals with the null genotype are unable to detoxify PAHs through this particular glutathione pathway. Although several epidemiological studies have found the null genotype to be associated with increased risk for the development of lung and other tobacco-related cancers (Hirvonen et al. 1993; Kihara and Noda 1994; Saarikoski et al. 1998), the findings in other studies are conflicting, and this association remains controversial (Zhong et al. 1991; Brockmoller et al. 1993).

## 13.8 Screening for Early Detection

The ideal method for decreasing the burden of lung cancer in the near and distant future is through primary prevention (i.e., decreasing the initiation of cigarette use), especially in children and adolescents. Nevertheless, there are more than 40 million current smokers who are at risk for the development of lung cancer, only some of whom will successfully stop smoking. Additionally, there are 40–50 million former smokers in the US who remain at an increased risk for the development of lung cancer even decades after quitting smoking (Hammond 1966; Doll and Peto 1976; Rogot and Murray 1980). Therefore, the development of effective lung cancer screening programs has been the focus of much interest in scientific research and public health domains. Early strategies for screening for lung cancer included the use of chest x-rays with or without cytologic analysis of sputum. More recent strategies have looked at the use of more sophisticated chest imaging and the incorporation of molecular biomarkers of lung carcinogenesis.

### 13.8.1 Preneoplasia and Intraepithelial Neoplasia

Risk of lung cancer among long-term heavy smokers continues even years after stopping smoking and is highest in smokers with chronic obstructive pulmonary disease. The prevalence of pre-invasive lesions did not change substantially for more than 10 years after cessation of smoking. Lung function was associated with the prevalence of pre-invasive

lesions (Lam et al. 1999). Numerous recent studies have indicated that lung cancer is not the result of a sudden transforming event in the bronchial epithelium but a multi-step process in which gradually accruing sequential genetic and cellular changes result in the formation of an invasive (i.e., malignant) tumor. Mucosal changes in the large airways that may precede or accompany invasive squamous carcinoma include hyperplasia, metaplasia, dysplasia, and carcinoma in situ (CIS) (Franklin 2000). Hyperplasia of the bronchial epithelium and squamous metaplasia are generally considered reversible and are believed to be reactive changes in the bronchial epithelium, as opposed to true pre-neoplastic changes (Wistuba et al. 1999). In contrast, moderate-to-severe dysplasia and CIS lesions seldom regress after smoking cessation (Lam et al. 1999) and frequently precede squamous cell carcinoma of the lung (Colby 1999). Advances in the understanding of lung cancer biology have led to observations that specific genetic changes occur in pre-malignant dysplasia (Kennedy et al. 1996).

### 13.8.2 Standard Chest X-Rays and Sputum Cytology

Lung cancer screening programs were first initiated in the early 1950s, around the time that the link between tobacco exposure and lung cancer was reported in the scientific literature. Since then, ten prospective trials have been designed and implemented that have utilized chest x-rays or sputum cytology in high-risk populations. Of note, nine of these trials did not include women. Three studies sponsored by the National Cancer Institute in the 1970s utilized a randomized trial design. Two of these studies, the Memorial-Sloan Kettering Study and the Johns Hopkins Lung Project, looked at the addition of cytology to chest x-ray screening (Melamed et al. 1984; Tockman 1986). While no benefit in terms of reduction of lung cancer mortality (considered the optimal endpoint due to absence of bias of screening efficacy) was gained with the addition of cytology, there were improvements in resectability and 5-year survival in the study population as compared to the Surveillance, Epidemiology and End Results database. The Mayo Lung Project (Fontana et al. 1984) evaluated chest x-ray plus cytology on an intensive schedule as compared to a control group, a portion of whom did receive chest x-rays on an annual or less frequent schedule outside of the study setting. There was no significant reduction in lung cancer mortality in the screened arm, and therefore the trial was originally deemed a negative screening trial. However, more recent analysis has shown that survival as an endpoint, which was improved in the screened arm, was not subject to biases (length, lead-time and overdiagnosis); as such, the reanalysis argues for a positive effect for this screening intervention (Strauss et al. 1997).

### 13.8.3 Helical Computed Tomography

A step forward in the development of lung cancer screening technologies is the low-dose helical or spiral computed tomography (CT) scan. Developed in the 1990s, this scan allows for x-ray scanning of the entire chest in approximately 15–25 s. Images approximating a

three-dimensional model of the lungs are generated via a computer program. Additionally, this technology employs a low dose of radiation and eliminates use of an intravenous contrast material, thus making it safer than the traditional high resolution contrasted CT scan. Limitations of this technology include a decreased sensitivity for detecting imaging abnormalities in the central regions of the lung, where more squamous cell lung cancers are located, and in the soft tissues of the middle of the thorax, where lymph nodes that may be involved by metastatic lung cancer are detected. Another potential limitation in the utilization of CT imaging in populations at risk for lung cancer is the detection of benign abnormalities, which may provoke unwarranted invasive and costly diagnostic procedures, such as biopsy.

A landmark study of the use of helical CT imaging in the screening of persons at risk for lung cancer has yielded important new data in lung cancer screening and early detection. The New York Early Lung Cancer Project (ELCAP) evaluated the usefulness of helical low-dose CT imaging in finding early stage lung cancers (Henschke et al. 1999). The ELCAP Study evaluated a non-randomized cohort of 1,000 smokers over the age of 60 and with a history of significant cigarette use with both annual chest x-rays and low-dose helical CT scans. The main outcome measure was the frequency of detection of non-calcified lung nodules by imaging technique. The superiority of helical low-dose CT scanning over chest x-ray in the detection of non-calcified lung nodules (found in 233 persons by CT compared with 68 persons by chest x-ray). By an algorithm that included the use of an additional high resolution CT scan to better assess the nodules and by assigning either a close follow-up program with re-imaging of the nodules versus proceeding directly to biopsy, spiral CT imaging detected close to six times more malignant nodules than did chest x-ray (2.3 versus 0.4%). Of 28 nodules biopsied, 27 were malignant; thus only one biopsy was performed for a benign nodule. Eighty percent of those lung cancers found by helical CT imaging were Stage I cancers (less than 3 cm in greatest dimension, without lymph node involvement or distant metastases), which have the highest potential for cure via surgical resection or radiation therapy. In general, chest x-ray detected the larger tumors. Of the 27 nodules detected by CT imaging, 26 were resectable. Importantly, the cost effectiveness of this strategy thus far has been impressive. The cost of a screening program consisting of a single baseline low-dose CT scan in a fit person at least 60 years of age with at least ten pack-years of smoking was only $2,500 per year of life saved (Wisnivesky et al. 2003). The cost of treating an early stage lung cancer is at least half the cost of treating an advanced stage lung cancer.

### 13.8.4 National Early Detection Initiatives

The provocative findings of the ELCAP study, in concert with re-analyses of earlier chest x-ray-based screening studies, have prompted a large screening initiative sponsored by the National Cancer Institute and the American Cancer Society. Initiated in 2002, the National Lung Screening Trial (NSLT), has reached its accrual goal of 50,000 current or former smokers who are randomized to receive annual screening with either spiral CT or traditional x-ray imaging for 3 years, followed by annual monitoring of participant health status

through 2009. Collection of relevant biologic samples (blood, urine and sputum) for use with diagnostic markers of lung cancer currently in development, as well as biomarkers of the carcinogenic pathway, is being conducted in a subset of participants (NCI 2004).

While these early data are suggestive of a benefit, in terms of decreasing the morbidity and mortality from lung cancer through screening for early lung cancers with CT imaging, there remains no clear consensus position with regard to use of imaging technologies for lung cancer screening; therefore, at this time, screening with CT or other imaging in current and former smokers is not considered a standard health care practice.

## 13.9 Chemoprevention

Prevention of lung cancer altogether is preferable to screening for early detection. The most effective means of preventing lung cancer is avoidance or elimination of tobacco use. Although the prevalence of cigarette smoking in the US has declined, the age-adjusted mortality of lung cancer has not shown a comparable decrease, partly due to the increased risk of lung cancer in former smokers. A central concept of chemoprevention is that intervention is likely to be most effective during identifiable pre-malignant steps of carcinogenesis. Lung cancer is an important public health concern in the US and the need for an effective chemopreventive agent against this cancer is tremendous.

At present, approximately 25% of the US adult population smokes. Primary prevention, through the reduction of cigarette smoking, is likely to be the most successful strategy in reducing lung cancer incidence. However, even if all current smokers in the US quit today, more than a million cases of lung cancer would develop over the next decade because of cigarettes already smoked (Wagner and Ruckdeschel 1995). Therefore, a strategy to prevent cancers before they develop is a critical element in reducing the burden of lung cancer.

### 13.9.1 Chemopreventive Agents Under Investigation

There has been explosive growth in the understanding of the molecular and genetic mechanisms underlying the carcinogenic process. This has allowed for identification of critical genes that may be mutated, silenced epigenetically, or overexpressed in pre-neoplastic and neoplastic tissue; additionally, key proteins, growth receptors and cellular pathways have been identified that may be aberrantly expressed in the carcinogenic pathway and/or invasive cancer. Consequently, agents have been rationally selected or designed to target these critical elements. Given that molecular targets relevant to cancer are often not highly nor aberrantly expressed in normal tissue, targeted agents often have good safety profiles for normal tissues and many agents currently in development have good oral availability. Listed in Table 13.2 are a number of molecular targets that been identified in the molecular pathogenesis of lung cancer; additionally, some representative targeted chemopreventive agents currently in development are listed. While this table lists agents that have been

developed synthetically, the target molecules or pathways have also been identified as targets of natural agents, such as botanicals and phytonutrients, that are being actively developed for lung cancer chemoprevention.

Various histological changes in the bronchial epithelium have been reported in association with chronic smoking and lung cancer. Furthermore, molecular changes have been found not only in the lungs of patients with lung cancer, but also in the lungs of current and former smokers without lung cancer. These observations are consistent with the multi-step model of carcinogenesis and "field cancerization" process, whereby the whole region is repeatedly exposed to carcinogenic damage (tobacco smoke) and is at risk for developing multiple, separate, clonally unrelated foci of neoplasia. The widespread

**Table 13.2** Chemopreventive agents under investigation for the prevention of lung cancer

| Molecular target/ pathway | Proposed role(s) in lung carcinogenesis | Targeted chemopreventive agent: Class (Representative agents) |
|---|---|---|
| Cyclooxygenase-2 (COX-2) | Product of arachdonic acid metabolism involved in tumor promotion-related events including cellular hyperproliferation, inhibition of programmed cell death (anti-apoptosis), new blood vessel formation (angiogenesis), inhibition of immune surveillance (Gridelli, Maion et al. 2000; Marks, Muller-Decker, et al. 2000) | Non-selective prostaglandin inhibitors (NSAIDs) Selective COX-2 inhibitors (Celebrex™) |
| Lipoxygenases | Enzyme products of arachidonic acid metabolism involved in growth-related signal transduction (Cuendet and Pezzuto 2000) | 5-, 8- and 12-lipoxygenase inhibitors |
| Ras protein farnesylation | The Ras protooncogene protein product requires farnesylation or related enzyme modification in order to relay signals for cell growth, differentiation, proliferation and survival. Mutated Ras is common in cancer and is associated with tumorigenesis. Other key proteins require farnesylation for function as well (Hahn, Bernhard, et al. 2001; Sebti 2003) | Farnesyl transferase inhibitors (Lonafarnib™, Zarnestra™) |
| Cyclin D1 | Regulatory molecule involved in cell cycle regulation; frequent aberrant expression of cyclin D1 resulting in unregulated cellular growth (Petty, Dragnev, et al. 2003) | Non-selective retinoids: all-trans-retinoic acid (ATRA); retinoid receptor selective agonists: (RAR) beta and retinoid X receptor (RXR) agonists |
| Epidermal Growth Factor Receptor (EGFR) | Cell transmembrane receptor; activation by growth factors causes signal transduction involved in tumor cell proliferation, anti-apoptosis, angiogenesis, and metastatic potential (Grandis and Sok, 2004; Yano, Kondo, et al. 2003) | EGFR inhibitors: Monoclonal antibodies (Erbitux®), tyrosine kinase oral inhibitors (Iressa™, Tarceva™) |

aneuploidy that occurs throughout the respiratory tree of smokers supports this theory. However, the presence of the same somatic p53 point mutation at widely dispersed preneoplastic lesions in a smoker without invasive lung cancer indicates that expansion of a single progenitor clone may spread throughout the respiratory tree. These molecular alterations might thus be important targets for use in the early detection of lung cancer and for use as surrogate biomarkers in the follow up of chemoprevention studies.

However, at present, no biomarker of lung carcinogenesis has been fully validated in a clinical trial using cancer as an endpoint. Because the multi-step process of carcinogenesis can take many years, assessment of clinical chemoprevention trials using cancer incidence as an endpoint requires lengthy follow-up period and large sample sizes. The use of surrogate endpoint biomarkers (SEBs) potentially circumvents these issues by evaluating a biologic event that takes place between a carcinogen or external exposure and the subsequent development of cancer. Because of field cancerization and the fact that the multi-path process of carcinogenesis is not regarded as a series of linear steps but rather as overlapping networks, multiple surrogate endpoint markers are preferable to identify potential epigenetic or genetic alterations leading to cancer. In order to be valid, the quantitative degree and pattern of the SEBs should correlate with carcinogenic transformation, respond to the intervention in a timely manner, and should reflect reversible events.

### 13.9.2 Dietary Supplements

In today's society, human activities and lifestyles generate numerous forms of environmental oxidative stress. Oxidative stress is defined as a process in which the balance between oxidants and antioxidants is shifted toward the oxidant side. This shift can lead to antioxidant depletion and potentially to biological damage if the body has an insufficient reserve to compensate for consumed antioxidants. The "antioxidant hypothesis" proposes that vitamin C, vitamin E, carotenoids, and other antioxidants in fruit and vegetables afford protection against heart disease and cancer by preventing oxidative damage to lipids and to DNA, respectively. Therefore, an increased oxidative stress accompanied by reduced endogenous antioxidant defenses may have a role in the pathogenesis of cancer.

The ability to evaluate any reduction in the primary endpoint of lung cancer requires large studies with large samples and lengthy follow up. The few phase III trials completed, including the Alpha-Tocopherol Beta Carotene Cancer Prevention Study (ATBC Study) (1994) and the beta-Carotene and Retinol Efficacy Trial (CARET) (Omenn et al. 1996), designed to prevent the occurrence of lung cancer in male current smokers, were negative. The CARET study evaluated beta-carotene and retinol in two populations at risk for lung cancer, including male asbestos workers and female and male cigarette smokers with 20 pack-years or greater history (either current or former smoker within 6 years of cessation). This was a randomized trial utilizing a 2 by 2 factorial design. An increase in lung cancer in the cohort receiving study vitamins was noted, although it did not reach statistical significance. With further analysis, it appeared that the beta-carotene supplemented cohort of current smokers had a 28% increase in rate of lung cancer (Omenn et al. 1996).

The surprising detrimental effect of beta-carotene in the CARET trial was in accordance with findings of another large 2 by 2 factorial design study in Finnish male smokers utilizing beta-carotene and vitamin E (ATBC Study). This study reported that the group taking beta-carotene supplements had an 18% increase in lung cancer as well as an 8% increase in total mortality (1994). There did not appear to be a benefit, in terms of lowering lung cancer risk, for α-tocopherol. It has been since hypothesized that oxidation products of beta-carotene formed in the presence of smoke may have procarcinogenic effects, with some in vitro and in vivo data supportive of this hypothesis (Wang and Russell 1999). These studies illustrate the importance of testing hypotheses derived from epidemiologic and laboratory data in the setting of large, randomized controlled trials, with careful consideration of potential and possibly unexpected interaction in the populations to be studied. Despite the less than encouraging results with retinoids in the earlier trials, there is continued interest in the development and testing of novel natural and synthetic retinoids as lung chemopreventive agents.

Results have been published from an intergroup phase III placebo-controlled, randomized trial in which 1,166 patients with pathological stage I NSCLC were treated with retinoid isotretinoin or placebo. Treatment did not improve the overall rates of second primary tumors, recurrences, or mortality (Lippman et al. 2001). Furthermore, secondary multivariate and subset analyses suggested that isotretinoin was in fact harmful to current smokers and beneficial only in never smokers. Despite the unexpected results from the CARET and similar supplementation trials showing that supplementation with micronutrients increased, rather than decreased, lung cancer incidence, considerable interest remains in investigating how other compounds in fruits and vegetables may affect lung cancer risk.

Chemopreventive treatments using other agents are currently being studied for the prevention of lung cancer. Vitamin E and aspirin are being evaluated in female nurses greater than 45 years of age in a randomized chemoprevention trial, using 9-cRA or 13-cRA and α-tocopherol or placebo (Kurie et al. 2003). This study is evaluating individuals at high risk with no previous history of lung cancer and may help to determine if active chemoprevention efforts can reduce the risk for developing aero-digestive cancer.

### 13.9.3 Selenium

Selenium is an essential mineral, though only in trace amounts. Overt and pathology-inducing selenium deficiency among humans is extremely rare except for parts of China. In most areas of the world, normal food consumption is adequate to saturate the selenoenzyme systems identified to date. Results from epidemiological studies, human clinical intervention trials, and in vitro and in vivo animal models clearly support a protective role of selenium against cancer development (Combs 1999; Nelson et al. 2002). Although selenium compounds have been shown to suppress carcinogenesis in many animal models and cell line systems, the mechanisms by which selenium may exert its chemopreventive activity still remain unclear.

El-Bayoumy (El-Bayoumy 2001) reviewed potential mechanisms for the protective role of selenium against cancer. Different forms of selenium have been used in rodents, at multiple organ sites including the lung, to test hypotheses including inhibition of carcinogen-induced covalent DNA adduct formation (Prokopczyk et al. 1996) and retardation of oxidative damage to DNA, lipids and proteins (Narayanaswami and Sies 1990). The effects of these forms of selenium on cell growth and molecular targets of carcinogenesis have been extensively studied in cell culture and animal systems. Tumor cell growth, DNA, RNA and protein synthesis, apoptosis, cell death, cell cycle, p53, AP-1, and nuclear factor kappa beta (NFκB), aberrant crypt foci (ACF), COX-2, protein kinase C and A (PKC and PKA), thymidine kinase (TK), jun-N-kinase (JNK), DNA cytosine methyltransferase, cell proliferation and cell cycle biomarkers and 8-isoprostane have demonstrated the ability to be modified by selenium treatment (Ronai et al. 1995; Wu et al. 1995; Fiala et al. 1998; Kawamori et al. 1998; Ip et al. 2000; Rao et al. 2000).

Ecologic studies suggest that dietary intakes of selenium are inversely associated with the risk of developing lung cancer. Shamberger and Frost (Shamberger and Frost 1969) were the first to report an inverse relationship between selenium levels in grain, forage crops, human blood and lung cancer mortality in regions of the US. Schrauzer (Schrauzer 1976) studied data from 27 countries and showed that dietary intake of selenium was inversely correlated with total cancer mortality as well as with age-adjusted mortality due to lung cancer. These findings were corroborated by population- based epidemiological studies in both the US (Clark 1985) and China (Yu et al. 1985). A number of case-control studies have examined selenium status in cancer patients compared to controls (Salonen et al. 1984; Nomura et al. 1987; Knekt et al. 1990; Criqui et al. 1991; Kabuto et al. 1994; Knekt et al. 1998). In most cases, a lower selenium status in cancer patients was reported, although this finding has not been consistent. Methodological issues such as the assessment of selenium exposure and the effects of treatment and disease stage on selenium status may explain some of these apparent inconsistencies. Many prospective studies of serum selenium levels and lung cancer risk have been published. Most of the studies used a nested case-control approach and two studies evaluated pre-diagnostic concentrations of selenium in toenail clippings and their association with lung cancer (Garland et al. 1995).

Knekt and coworkers (Knekt et al. 1998) found a significant inverse association between serum selenium and subsequent lung cancer occurrence in men within the cohort studied in the Finnish Mobile Health Examination Survey. However, this study showed no inverse association between reported selenium intake and lung cancer risk (Knekt et al. 1991). A strong inverse association between toenail selenium and lung cancer in men and women was observed in a longitudinal observational study from the Netherlands (van den Brandt et al. 1993). Other published studies suggested inverse trends in lung cancer risk with increasing selenium status but were non-significant due to small numbers of cases. Conversely, non-significant positive associations between serum selenium and lung cancer risk have been observed (Menkes et al. 1986). Garland and coworkers (Garland et al. 1995) reported significantly lower toenail selenium levels among lung cancer case patients compared with control subjects; however, control for smoking reversed this association. Methodological issues, including the use of toenail selenium, must be considered.

Northern Italy provided an uncontrolled experiment of selenium exposure and subsequent mortality for cancer in humans. A statistically non-significant lower risk of lung cancer was

detected in females residing in the Italian community exposed to selenium through drinking water (Vinceti et al. 1995). Conversely, males exposed through drinking water had a non-significantly increased risk (Vinceti et al. 1995). Overall, observational data tend to show a statistically non-significant inverse association between selenium levels and lung cancer.

### 13.9.4 Tea and Derivatives

Tea is a beverage made from the leaves of *Camellia sinensis* species of the Theaceae family. This beverage is one of the most ancient and, next to water, the most widely consumed liquid in the world. Tea leaves are primarily manufactured as green or black or oolong, with black tea representing approximately 80% of the tea products consumed. Green tea is the non-oxidized, non-fermented product of the leaves and contains several polyphenolic components, such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). EGCG is the major green tea polyphenol (more than 40% dry weight). The lowest effective dose of 0.016 mmol EGCG/kg per day in rodent cancer models is comparable to the consumption of four cups of green tea or 17.7 mmol/kg per day of EGCG by a 70 kg man (1996).

Tea polyphenols are the major polyphenolic compounds of tea. They scavenge active oxygen radicals (Cheng 1989) and inhibit DNA biosynthesis of the tumor cells (Katiyar et al. 1992) and chemocarcinogen-induced carcinogenesis (Xu et al. 1993). They also block the inhibition effect of carcinogens in intercellular communication (Sigler and Ruch 1993) and induce apoptosis (Zhao et al. 1997). Tea-derived polyphenols exhibit antimutagenic and genotoxic activities that may be associated with anticarcinogenic activity (Okai and Higashi-Okai 1997). Stich et al. (Stich et al. 1982) showed that water-soluble extracts of green and black teas inhibited the mutagenicity in a nitrosation model system.

Because cigarette smoking and tea drinking are very common in many diverse populations, several studies have explored the possible inhibitory effects of tea on lung cancer formation induced by cigarette smoking. Several studies have reported that green tea and black tea inhibit the formation of lung tumors in A/J mice induced by the tobacco-specific nitrosamine 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK), the most potent carcinogen found in cigarette smoke (Wang et al. 1992; Xu et al. 1992). The effect of tea may be related to the significant inhibitory effects on NNK-induced oncogene expression in mouse lung (Hu et al. 1995).

Tea is a promising agent for the potential chemoprevention of cancer (Yang and Wang 1993; Stoner and Mukhtar 1995). Polyphenolic compounds present in tea afford protection against chemical carcinogen-induced tumor initiation and tumor promotion in lung and forestomach of A/J mice (Wang et al. 1992; Xu et al. 1992). A recent study investigated the effects of oral administration of decaffeinated green tea or black tea on NNK-induced lung tumorigenesis (Yang et al. 1998). Significant protection against lung tumor formation occurred when tea was given either during or after NNK treatment. A study on the bioavailability of radioactive EGCG revealed that radioactivity was widely distributed into various organs of mouse and that 0.16% of total administered radioactivity was observed in lung tissue 24 h after oral administration (Suganuma et al. 2001).

Ohno et al. (Ohno et al. 1995) showed that daily tea consumption significantly decreased the risk of lung squamous cell carcinoma in males and females; the odds ratios were 0.50 and 0.8, respectively. Mendilaharsu et al. (Mendilaharsu et al. 1998) investigated the effect of drinking tea on the lung cancer risk of male cigarette smokers in a case-control study in Uruguay. They found that high intake (two or more cups per day) was associated with a reduced risk of lung cancer in smokers (0.34; 95% confidence interval [CI]: 0.14–0.84). Flavonoids, including catechins, have been reported to protect against chronic lung disease. Total antioxidant capacity of plasma was significantly increased after taking green tea in amounts of 300 and 450 ml and a positive increment according to green tea dosage was also observed (Sung et al. 2000). A Japanese prospective cohort study revealed that the consumption of ten cups (120 ml each) of green tea per day delayed cancer onset of both never smokers and current smokers. Green tea showed the strongest protective effects on lung cancer (relative risk = 0.33) (Fujiki et al. 2001). However, results have not been consistent; Tewes and colleagues (Tewes et al. 1990) reported a tentative increase in lung cancer risk among green tea drinkers. Results were stated as tentative since only 23 cases (11.5%) and 13 controls (6.5%) reported regular tea drinking and the authors did not have data to perform a dose-response analysis.

Investigations into the anticarcinogenic properties of tea (studies of green tea and green tea extracts) have shown growth inhibitory effects in a number cancer cell lines (Ahmad et al. 1997; Suganuma et al. 1999). Investigation into the mechanism of EGCG-induced apoptosis revealed that treatment with EGCG resulted in DNA fragmentation, induction of caspase-3/CPP32 activity, and cleavage of the death substrate poly(ADP-ribose)polymerase (Islam et al. 2000). Masuda and co-workers (Masuda et al. 2001) examined the molecular effects of EGCG on two human head and neck SCC cell lines, YCU-N861 and YCU-H891, focusing on the EGFR signaling pathway. Treatment with EGCG induced apoptosis and caused a decrease in the Bcl-2 and Bcl-X(L) proteins, an increase in the Bax protein, and activation of caspase 9, suggesting that EGCG induces apoptosis via a mitochondrial pathway. Treatment with EGCG also inhibited phosphorylation of the EGFR and also inhibited basal and transforming growth factor-alpha-stimulated c-fos and cyclin D1 promoter activity. EGCG at 0.1 mcg/ml (a concentration found in serum after oral administration) enhanced the growth-inhibitory effects of 5-fluorouracil. Taken together, these findings provide insights into molecular mechanisms of growth inhibition by EGCG.

## 13.10 Conclusion

In the New Millennium, lung cancer continues to exact an extremely large toll in terms of cancer-related morbidity and mortality as well as its effect on health care systems and economies worldwide. This is in spite of public health efforts initiated in the 1960s to educate the public about the dangers of tobacco use and the implementation of smoking cessation programs. While up to 50–60% of adults in some countries continue to smoke, formers smokers are growing in number. Unfortunately, this latter population remains at elevated risk for developing lung cancer.

A growing understanding of lung carcinogenesis from the molecular and genetic standpoint has complemented epidemiologic studies that have identified high-risk populations that are most likely to benefit from intervention strategies. Chemoprevention strategies currently employ a wide range of selective chemopreventive approaches that include dietary modification and supplementation using natural products and their derivatives, as well as a range of synthetic agents that may also have utility in treating advanced lung cancer. Complementing chemoprevention efforts is a growing body of research that suggests that state-of-the-art screening techniques for early detection will impact on the morbidity and mortality associated with lung cancer.

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# Breast Cancer Prevention

# 14

P.A. Thompson, A.T. Stopeck

## 14.1 Introduction

Early detection, combined with targeted and more effective therapies, has led to reductions in breast cancer related deaths. Approximately 90% of women diagnosed with breast cancer in the US today are disease free 5 years after diagnosis. Despite these successes, breast cancer remains a major cause of death, particularly among young women. In addition, chronic or prolonged toxicities associated with cancer treatments contribute to overall morbidity and reduced quality of life for more than two million breast cancer survivors in the US alone. Increasing rates of breast cancer worldwide, especially among previously low-risk regions and low-risk populations, are creating new concerns (Minami et al. 2004; Althuis et al. 2005; Bosetti et al. 2005). This is a particularly worrisome trend for regions and populations where use of screening and access to targeted therapies are limited. Given the worldwide burden of breast cancer in terms of lives impacted (nearly 1.3 million new cases per year) and lives lost annually (estimated 464,854 annual deaths), prevention of invasive breast cancers remains a major global public health priority (Garcia et al. 2007).

In this chapter we update information on breast cancer etiology and changing patterns of disease incidence by race and age with a focus on female breast cancers. Male breast cancer is rare and distinct from breast cancers that occur among women and are discussed elsewhere (Dimitrov et al. 2007; Tai et al. 2007). This chapter details current information on risk factors, emphasizing their contribution to risk assessment tools and clinically useful definitions of high risk for patient counseling. We address race and breast cancer with a focus on what the physician needs to know about screening and risk assessment in specific populations. For example, the status on the efficacy of available genetic testing for familial forms of the disease and risk assessment tools are described as they apply to African American, Asian and Hispanic women. This chapter concludes with a discussion of current thinking regarding the role of prevention strategies for women at low, moderate

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and high risk for breast cancer, with special focus on current recommendations for the use of the selective estrogen receptor (ER) modulators or SERMs (e.g., tamoxifen, raloxifene) for the primary prevention of disease in women at high risk.

## 14.2 Etiology

The current understanding of breast tumorigenesis suggests that invasive cancers arise through a series of molecular alterations that manifest at the cell level, ending in the outgrowth and spread of breast epithelial cells demonstrating immortal features and uncontrolled growth (Lacroix et al. 2004). Perhaps the most significant advance in our understanding of breast tumorigenesis comes from the extensive genomic efforts that have demonstrated the presence of discrete breast tumor subtypes with distinct clinical behavior (e.g., five subclasses: luminal A, luminal B, basal, ERBB2+, and normal breast) (Sorlie et al. 1994; Perou et al. 2000; Sorlie 2004). The exact number of disease subtypes and molecular alterations from which these subtypes derive remains to be fully elucidated, but they generally align closely with the presence or absence of hormone receptor and mammary epithelial cell type (luminal or basal). Figure 1 summarizes the current general understanding of breast tumor subtypes, prevalence and the major associated molecular alterations. This view of breast cancer, not as a set of stochastic molecular events, but as a limited set of 'separable' diseases of distinct molecular and cellular origins, has altered thinking about breast cancer etiology, type-specific risk factors, and prevention strategies. Given the apparent significance of the tumor subtypes in terms of clinical appearance and behavior, we have attempted to comprehensively include available data by subtype classification, integrated into the current breast tumor classification schema, in the context of prevention and risk assessment.

## 14.3 Changing Patterns of Breast Cancer Incidence

The lifetime risk of breast cancer is now estimated at 12.7% for all women, 13.3% for non-Hispanic Whites and 9.98% for African American women (Smigal et al. 2006). With the introduction and uptake of mammography-based screening, a dramatic increase in the incidence of breast cancer was observed and peaked in the 1980s, particularly among women between the ages of 50 and 69 years. This increased incidence coincided with a doubling of the number of small tumors (less than 2 cm) and a reduced rate of increase in the incidence of advanced disease by as early as 1987 (Garfinkel et al. 1994). After 1987, overall rates of invasive breast cancers continued to rise but at a much slower rate, specifically among White women age 50 or older (increased only by 0.5% per year).

For specific histological types, rates varied dramatically. Common ductal carcinomas showed a modest 3% increase from 1987 to 1999, while invasive lobular carcinomas and

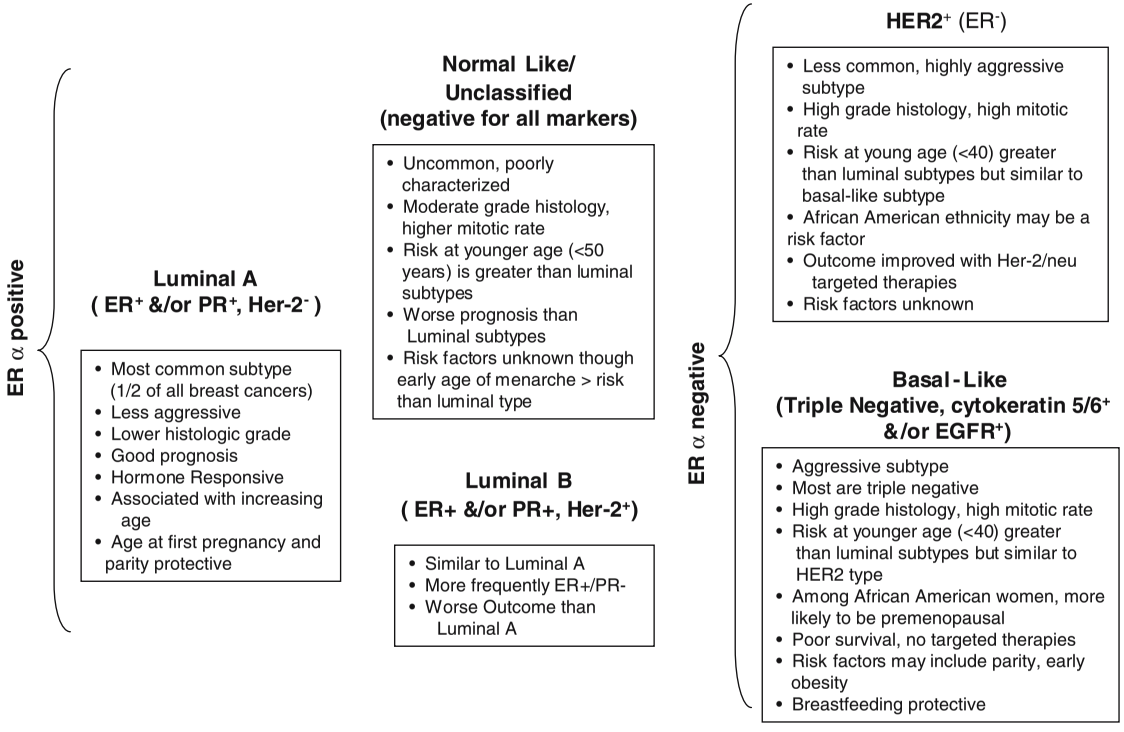


**Fig. 1** Intrinsic subtypes of breast cancer

mixed ductal-lobular increased 52% and 96%, respectively (Li and Daling 2007). Overall, the annual incidence rates in African American (119.4 out of every 100,000) and Hispanic/Latina (89.9 out of every 100,000) women have been stable since the early 1990s and have an overall lower annual incidence of breast cancer than White women (141.1 out of every 100,000). Incidence rates for women under the age of 50 have remained stable since the mid to late 1980s, with rates declining in African American women under the age of 50 as early as 1991. This declining rate has been suggested to reflect higher early age obesity rates (< 18 years) associated with the recognized lower risk for premenopausal breast cancer, though the mechanism is suspected to reflect reduced ovarian function and an ovulation in obese young women, the mechanism is unknown (Huang et al. 1997; Palmer et al. 2007). A similar protective effect of early onset obesity might explain decreasing rates among American Indians and Alaska Natives (decreasing by approximately 3.7% per year) (Smigal et al. 2006). This is in contrast to rates among Asians and Pacific Islanders, which have continued to increase at 1.5% per year. In spite of lower overall rates when compared to Whites, African American women remain more likely than White women to be diagnosed with large, advanced stage tumors (greater than 5 cm).

Between 2001 and 2004, breast cancer rates in the Surveillance, Epidemiology, and End Results (SEER) Program data show a dramatic 8.6% decrease in invasive disease across all age groups, an 11.8% annual decline for all cancer types, and a 14.7% decline for ER positive cancers among women 50–69 years of age (Ravdin et al. 2007). During this same time period, no significant change was observed in the incidence of ER negative cancers or cancers in women under age 50. This decrease in new cases in the US is expected to continue with approximately 178,480 new invasive breast cancers and an estimated 40,910 deaths from breast cancer each year estimated per year in the next future. (Jemal et al. 2007). The decline in rates among older women has been limited to non-Hispanic whites and has not been observed in other racial or ethnic groups (Glass et al. 2007; Jemal et al. 2007; Katalinic and Rawal 2007; Ravdin et al. 2007). This significant and dramatic decline in incidence has sparked considerable debate as to the underlying cause or causes explaining these changes. Given the sharp and near immediate decline in rates coincident with the July 2002 publication of the Women's Health Initiative (Writing Group for the Women's Health Initiative, 2002) on the risks associated with combined estrogen and progestin postmenopausal hormone replacement therapy (HRT) use, significant attention has been directed at the influence of reduction in estrogen and progestin HRT use on breast cancer incidence rates (Ravdin et al. 2006). While a variety of other factors may have contributed to the observed reduction, a recent study (Kerlikowske et al. 2007b) showed a similar 13% decline in estrogen receptor (ER) positive tumors in women age 50–69 years, supporting the relationship between reductions in incidence with decreased HRT use.

To further examine the reasons for declining rates of breast cancer in the US, Jemal and colleagues evaluated data from the nine oldest SEER cancer registries dating back to 1975 (Jemal et al. 2007). Two trends in incidence patterns were observed. First, age-specific incidence rates declined in all women 45 and older beginning as early as 1999. This was subsequently supported by a study on rates, ethnicity and histologic subtypes using 13 of the SEER population-based cancer registries with rates for both ductal and lobular types of invasive breast cancer (Li and Daling 2007). Both Jemal and Li

argue that the declining rates are in part explained by a 'saturation' in use of screening mammography, of the target population giving rise to an expected plateau in incidence at or near the late 1990s when the adoption of mammogram-based screening stabilized at 70% use (Jemal et al. 2007; Li and Daling 2007). This saturation in uptake has resulted in a reduction in the pool of previously undiagnosed prevalent cases leading to, declines in new diagnoses. In particular, this appears to be the case for women older than 69 years of age, whose rates of breast cancer began to show evidence of decline as early as 1998 when screening rates had first reached a plateau. This observation is also consistent with the prediction that with widespread screening and earlier detection, breast cancer rates are likely to peak among women during the sixth and seventh decades of life and then decline; exactly the incidence pattern that is now reported for screened populations (Anderson et al. 2007).

Jemal and colleagues further describe how the plateau effect alone cannot explain the observed age differences in the magnitude and years of observed declines in rates. For example, women 60–69 years of age experienced a dramatic rate decrease from 2002 to 2003. However, women of other ages experienced a similar decline during this same time period. Breast cancer rates among women between the ages of 55 and 59 decreased by 11.3%, rates decreased by 10.6% among women 60–64 years of age, and decreased by 14.3% among women 65–69 years of age (Glass et al. 2007; Katalinic and Rawal 2007; Kerlikowske et al. 2007b; Ravdin et al. 2007). It is important to note that a significant decline was only observed among ER positive and progesterone receptor (PR) positive tumors. Women 65–69 years of age experienced a 20% reduction in $ER^+$ and $PR^+$ tumor compared to a 2% increase in $ER^-$ and $PR^-$ tumors. The current evidence favors the hypothesis that removal of exogenous hormones (e.g. HRT) may slow or even stop the growth of a present, but as yet undetected lesion. It remains to be determined if this reduction in exposure to exogenous hormones will be sufficient to sustain a decrease in breast cancer rates or if this reflects a slowing or delaying in the detection process. It is too early to assess whether or not these declines are temporary or will be sustained with secular changes in the use and prescribing patterns of HRT.

These data continue to support the large body of evidence that postmenopausal hormonal exposure (exogenous and endogenous) increase the risk of hormone responsive tumors (Chen et al. 2004; Rosenberg et al. 2006; Santen et al. 2007). These observations favor reductions in hormone exposures, to reduce the risk of hormone receptor positive tumors that occur with advancing age. Lifestyle and pharmacologic strategies emphasize the need to tailor use of HRT to the individual patient and her specific needs.

## 14.4 Established Breast Cancer Risk Factors

There are several risk factors clinically useful for assessing a patient's risk for breast cancer (Table 14.1). Many of these factors form the basis of breast cancer risk assessment tools that are currently available.

**Table 14.1** Established breast cancer risk factors

| Risk factor | Estimated relative risk |
|---|---|
| Advanced age | >4 |
| Family history | |
| Two or more relatives (mother, sister) | >5 |
| One first degree relative (mother, sister) | >2 |
| Family history of ovarian cancer <50 | >2 |
| Personal history | |
| Breast cancer | 3–4 |
| Positive for BRCA1/BRCA2 mutation | >4 |
| Breast biopsy with atypical hyperplasia | 4–5 |
| Breast biopsy with LCIS or DCIS | 8–10 |
| Reproductive history | |
| Early age at menarche (<12) | 2 |
| Late age of menopause | 1.5–2 |
| Late age of first term pregnancy (>30) nulliparity | 2 |
| Use of combined estrogen/progesterone HRT | 1.5–2 |
| Current or recent use of oral contraceptives | 1.5 |
| Lifestyle factors | |
| Adult weight gain | 1.5–2 |
| Sedentary lifestyle | 1.3–1.5 |
| Alcohol consumption | 1.5 |

### 14.4.1 Age, Gender and Breast Cancer Risk

Increasing age and female sex are established risk factors for breast cancer. Sporadic breast cancer is relatively uncommon among women younger than 40 years of age but increases significantly thereafter. Among screened populations, incidence rates appear to be highest between 50 and 69 years of age, followed by a leveling off or even decline with age after 70 (Anderson et al. 2007). The significant shift in incidence rates during the years surrounding menopause is the basis for initiating annual mammography screening for all women at age 40. The efficacy of screening the general population under age 40 remains unclear due to low rates of breast cancer and the reduced sensitivity of current screening methods in younger women (Smith 2000; Buist et al. 2004). The effect of age on risk is further illustrated in the SEER data where the incidence rate of invasive breast cancer for women under the age of 50 years is 42.5 per 100,000 as compared to 375 per 100,000 for women 50 years of age or older (Smigal et al. 2006). The total and age-specific incidence for breast cancer appears to be bimodal, with the first peak occurring at about 50 years of age and the second occurring at about 70 years of age. This bimodal pattern may reflect the influence of age within the different tumor subtypes with prevalence of poorly differentiated, high grade disease occurring earlier compared to hormone sensitive, slower growing

tumors that occur with advancing age (Nixon et al. 1994; Wildiers et al. 2007). These results however remain controversial with incidence data between 1985–1989 and 1998–2002 from the National Cancer Institute and the North American Association of Central Cancer Registries suggesting that postmenopausal breast cancer rates peak between 75 and 79 years and decline thereafter secondary to lower screening rates in the advanced elderly (Smigal et al. 2006). These data support the use of screening mammography as women age taking into consideration individual health status and estimated life expectancy. The 2003 update to the American Cancer Society (ACS) breast cancer screening guidelines (Table 14.2) recommends that physicians continue to use mammography as a primary tool to detect breast cancer among women who would be candidates for cancer treatment, regardless of their age (Smith et al. 2003).

### 14.4.2 Family History of Breast Cancer

A positive family history of breast cancer is the most widely recognized risk factor for breast cancer. Risk is approximately five times greater in women with two or more first-degree relatives with breast cancer and is also greater among women with a single first-degree

**Table 14.2** Breast cancer early detection guidelines by risk category and age[a]

| Age | Average and moderate risk | High risk |
|---|---|---|
| 20s and 30s | Monthly breast self exam (BSE) following instruction | BSE and CBE as recommended in average risk women |
| | Clinical breast exam (CBE) as part of regular health exams, minimum every three years | Consideration of earlier initiation of screening methods with shorter screening intervals |
| | Awareness to report any change in breast health | Consideration of other screening modalities to include ultrasound and magnetic resonance imaging methods |
| | | Heighten awareness to report any change in breast health |
| 40s through 60s | Continuance of BSE, CBE and introduction of mammography screening | Vigilance in patient surveillance and consideration of shorter intervals for screening |
| | Heighten patient awareness to report any change in breast health | |
| Over age 70 | Consideration of overall health benefit with mammography in context of health status and expected longevity | Continuance of surveillance vigilance in context of expected patient longevity |

[a]Modified from Smith, Saslow et al., The ACS Guidelines for Early Breast Cancer Detection, 2003

relative, particularly if diagnosed at an early age (age 50 or younger). A family history of ovarian cancer in a first-degree relative, especially if the disease occurred at an early age (younger than age 50), has been associated with a doubling of risk of breast cancer. In 5–10% of familial cancer cases, risk is inherited as an autosomal dominant disorder. These hereditary cancers represent a distinct subset of familial breast cancers, which exhibit high penetrance and clustering with ovarian cancers (Antoniou et al. 2003). The *BRCA1* and *BRCA2* gene mutations, on chromosome 17 and 13, respectively, account for the majority of autosomal dominant inherited breast cancers (Narod and Foulkes 2004). Mutation rates may vary by ethnic and racial groups (John et al. 2007). For *BRCA1* mutations, the highest rates occur among Ashkenazi Jewish women (8.3%) followed by Hispanics (3.5%), non-Hispanic Whites (2.2%), African Americans (1.3%), and Asian Americans (0.5%). Women who inherit a mutation in the *BRCA1* or *BRCA2* gene suffer an estimated 50–80% lifetime risk of developing breast cancer (Antoniou et al. 2003).

To aid the clinician in the identification of mutation carriers of *BRCA1/2*, a number of family history based risk assessment tools have been developed. These include BRCAPRO, Couch, Myriad I and II, Ontario Family History Assessment Tool (FHAT), and the Manchester models. All of these assessment tools are highly predictive of carrier status and aid in reducing testing costs for the majority of mutation negative families (Parmigiani et al. 2007). The most commonly used BRCAPRO model identifies approximately 50% of mutation negative families, avoiding unnecessary genetic testing, while only failing to identify 10% mutation carriers for screening. It is important to note that these clinical genetic risk assessment tools have been developed from mutation rates in Ashkenazi Jewish families and families of European descent. However, these models have been evaluated for their applicability to other populations and have proven to be robust for African American and for Hispanic women. Age of onset and the number of affected family members with breast or ovarian cancer are the most powerful predictors of mutation carrier status (Nanda et al. 2005; Vogel et al. 2007). Thus, these tools are applicable for use in screening all women with strong family histories for possible *BRCA* mutations.

For health care providers, it is notable that a significant portion of ovarian cancers, not previously considered familial, can be attributed to *BRCA1* or *BRCA2* mutations (Tuya Pal et al. 2005). In one study of 209 women with invasive ovarian carcinoma not previously identified as familial, 15.3% had mutations in *BRCA1* or *BRCA2* (20 BRCA1 and 12 BRCA2 mutations. None of the ovarian cancers positive for *BRCA1* or *BRCA2* mutations were of the lower risk borderline or mucinous histologies. These data, though limited, have led to the suggestion that women with non-mucinous, invasive ovarian cancers may benefit from genetic testing to determine mutation status independent of a family history of breast cancer. Confirmation of this high BRCA carrier rate among ovarian cancers is needed to change current BRCA testing.

Inherited mutations in *BRCA1* and *BRCA2* genes account for only 5–10% of all breast cancers but are responsible for the majority of familial breast cancers. Among patients with strong family histories who initially test negative for *BRCA1* and *BRCA2*, an additional 12% of cases may carry more complex genomic deletions or duplications in either *BRCA1* or *BRCA2* with nearly 5% carrying mutations in a third cell-cycle checkpoint kinase gene (CHEK2) or mutations in the tumor suppressor gene TP53 (Walsh et al. 2006). Three additional genetic conditions have been associated with a high risk of early onset

breast cancer (Hodgson et al. 2004). These include Li–Fraumeni syndrome, Cowden syndrome and Peutz-Jeghers syndrome. These rare syndromes are caused by mutations in TP53, PTEN and LKB1, respectively.

Even in families without documented high-penetrance mutations, breast cancer in a single first degree relative and even in a second degree relative confers a two to 3-fold increase in risk (Culver et al. 2006). Thus, genetic susceptibility is suspected in 25% of all breast cancer cases and may contribute to a portion of apparent sporadic disease through a mode of inheritance thought to be more complex than a single dominant acting gene (Peto and Mack 2000; Stratton and Rahman 2008). For example, a specific variant in the CHEK2 gene designated 1100delC that occurs in the heterozygous state in about 0.5–1% of the population has been associated with between a three- and five-fold increased risk for breast cancer in the general population and a risk approximating that of *BRCA1* mutation carriers for women at age 70 years or more (Weischer et al. 2008). Currently, a genetic test for the CHEK2 gene deletion is available through the City of Hope Clinical Molecular Diagnostic Laboratory.

In contrast to the CHEK2 gene, a number of similar direct-to-consumer tests are rapidly emerging for newly identified modifier genes of undetermined significance (e.g. variants on chromosomes 2 and 8, FGFR2, the TNRC9/TOX3, the LSP1, the MAP3K1 and the CASP8 genes). While variants in these newly identified genes may occur in 28–46% of breast cancer patients, they confer very weak to modest effects on risk (Easton et al. 2007; Hunter et al. 2007). Therefore, it is "premature to recommend screening women for these gene variants" (Hunter et al. 2007).

Physicians should be aware of the growing availability of gene-based tests (Hudson et al. 2007). Patients suspected to be at risk for hereditary forms of breast cancer should be advised to seek expert genetic counseling. It is anticipated that a number of genetic tests and information are likely to emerge in the near future, especially as large-scale genomic studies are in progress to identify important risk modifier genes akin to the CHEK2 1100delC. While it is anticipated that genetic information will ultimately improve individual risk assessment, premature delivery of unconfirmed gene-risk associations to the public carries potential to negatively impact the medical and patient community. To identify a genetic counseling center in your area, the National Institutes of Health (NIH) provides a partial listing of services at the Cancer Genetics Services Directory (http://www.cancer.gov/search/geneticsservices/).

### 14.4.3 Reproductive Risk Factors

Late age at first pregnancy, nulliparity, early onset of menses, and late age of menopause have all been consistently associated with an increased risk of breast cancer (Pike et al. 2004; Kelsey and Bernstein 1996; Colditz and Rosner 2000; Deligeoroglou et al. 2003; Colditz et al. 2004). Among the reproductive risk factors, women who experience natural menopause after 55 years of age have about twice the risk of breast cancer compared to women who experience natural menopause before age 45 (Trichopoulos et al. 1972). The observed increased risk among women with early menarche (younger than 12 years

of age) appears to be strongest in the premenopausal period, whereas risk of later onset breast cancer appears to be greater among women who experience a late first full-term pregnancy. Women who bear their first child after the age of 30 have twice the risk of developing breast cancer as compared to those who experience first full-term pregnancy before 20 years of age. Meta-analysis results suggest that each birth is associated with an 11% decrease in risk of developing $ER^+/PR^+$ cancer with no effect observed for $ER^-/PR^-$ tumors (Ma et al. 2006a,b). In contrast, breastfeeding and late age at menarche decreased the risk of both subtypes of breast cancer with late age of menarche more strongly protective for $ER^+PR^+$ cancers (Ma et al. 2006a,b). At present, the effect of reproductive factors among *BRCA* mutation carriers and women with a family history is unclear. There may be differing effects of reproductive factors among *BRCA1* as compared with *BRCA2* mutation carriers. Among *BRCA1* carriers, breastfeeding for 1 year and late age at first pregnancy are associated with a reduced risk of breast cancer (Jernstrom et al. 2004). This contrasts with *BRCA2* mutation carriers where late age at first pregnancy is associated with higher risk of breast cancer (Andrieu et al. 2006). These data are consistent with emerging evidence that support differential effects of reproductive risk factors on tumor subtypes and differences among tumor histologies that arise in *BRCA1* mutation carriers, who are at risk of breast cancers that are more likely to be high-grade, ER, PR and HER-2/neu triple negative or basal-like tumors (Lakhani et al. 2005). *BRCA2* mutation carriers are at risk of breast cancers that are more likely to be, $ER^+/PR^+$ and HER-2/neu negative (luminal type) (Bane et al. 2007).

### 14.4.4 Endogenous Hormone Exposures

Prolonged exposure to elevated levels of sex hormones, most principally estradiol (E2), has long been postulated as a significant factor for developing breast cancer, explaining the association between breast cancer and reproductive behaviors (Hankinson and Eliassen 2007). The results of numerous clinical trials demonstrate the protective effect of selective estrogen receptor modulators (SERMs) and aromatase inhibitors on recurrence and the development of contralateral breast cancers (Early Breast Cancer Trialists' Collaborative Group, 2005; Howell et al. 2005). Use of SERMs in individuals at increased risk of breast cancer has been demonstrated to prevent invasive $ER^+$ cancers (Fisher et al. 1998; Fisher et al. 2005; Vogel et al. 2006). These data support E2 and its receptor as a primary target in risk reduction strategies, but do not provide evidence that circulating hormone levels predict susceptibility. The National Surgical Adjuvant Breast and Bowel Project Cancer Prevention (NSABP) P-1 trial did not find any association between the efficacy of tamoxifen and the levels of circulating E2, testosterone, or sex hormone binding globulin (Beattie et al. 2006). While it has been argued that participants in the NSABP P-1 trial were enriched for risk and not representative of the general population (Beattie et al. 2006), it has been difficult to reconcile the lack of association between hormone levels and risk if indeed circulating hormone levels accurately inform on tissue exposures and tissue susceptibility. A number of issues have plagued assessment and interpretation of serum hormone levels in women including sampling issues, poor lab-to-lab and assay-to-assay correlation,

and general methodological issues of inadequate sensitivity and reliability (Santen et al. 2007). A number of recent epidemiologic and pooled studies (Santen et al. 2007; Key et al. 2002) support an elevated risk among women with high E2 levels. For example, the Endogenous Hormones and Breast Cancer Collaborative Group (EHBCG) reported a relative risk of 2.58 (95% confidence interval, CI: 1.76–3.78) among women in the top quintile of E2 levels. Upon thorough review of the collective data, the newly formed Breast Cancer Prevention Collaborative Group (BCPCG), which included authors of the study of serum hormones in the P-1 trial, favor prioritizing measures of plasma hormone levels (particularly free plasma E2 levels) as an additional component of existing tools to improve risk assessment for the individual woman (Santen et al. 2007). Others in the group favor limiting the assessment of plasma hormones to women not taking hormone therapy as risk associated with exogenous hormone therapy use, especially the combined forms, is well established. At present, the routine measurement of plasma hormone levels is not recommended in the assessment of breast cancer risk.

### 14.4.5 Exogenous Hormone Exposures

One of the most widely studied factors in breast cancer etiology is the use of exogenous hormones in the form of oral contraceptives (OCs) and HRT (Garbe et al. 2004). Current use of OCs has been inconsistently associated with a slight increased risk of breast cancer (1996; Marchbanks et al. 2002). The overall evidence suggests an approximately 25% greater risk of breast cancer among current users of OCs. The risk appears to decrease with age and time since OC discontinuation. Risk returns to that of the average population risk after approximately 10 years following cessation of note, among. Families at increased risk of breast cancer, OC use reduces the risk of ovarian cancer by 40–50% (McLaughlin et al. 2007). It remains unclear if the risk of breast cancer is increased or decreased with OC use among *BRCA1/2* mutation carriers (Brohet et al. 2007; Milne et al. 2005). At present, women at increased genetic risk should be evaluated on an individual basis regarding OC use, taking into consideration the prevalence of ovarian cancer in the family history with potential risks (slight elevation in risk of breast cancer) and benefits (significant protection for ovarian cancer) of OC use.

Consistent epidemiologic data (Cancer 1997; Schairer et al. 2000) support an increased risk of breast cancer incidence and mortality (Collaborators 2003) with the use of postmenopausal HRT. Risk is directly associated with length of exposure with greatest risk observed for the development of hormonally-responsive lobular (relative risk, RR=2.25, 95% confidence interval, CI: 2.00–2.52), mixed ductal–lobular (RR=2.13, 95% CI: 1.68–2.70), and tubular cancers (RR=2.13, 95% CI: 2.16–3.28) (Reeves et al. 2006). Risk is greater among women taking combination estrogen plus progestin formulations than estrogen-only formulations (Cancer 1997; Schairer et al. 2000). Published results of a randomized trial, the Women's Health Initiative (WHI), of estrogen-only and combination-HRT for the prevention of chronic disease indicate that the adverse outcomes associated with long-term use outweigh the potential disease prevention benefits particularly for women greater than 65 years of age (Anderson et al. 2004). Invasive breast cancer was

increased by 26% in all women randomly assigned to combination HRT when compared to placebo (Anderson et al. 2004). In contrast, breast cancer risk among the estrogen-only users in the WHI trial (Anderson et al. 2004) was associated with a slight but not significant decrease in breast cancer risk when compared to placebo, though follow up was relatively short due to early stopping of the trial. Data from large observational studies with longer follow up do demonstrate elevated risk for estrogen-only use, but at a consistently lower magnitude than that associated with combined HRT use (Collaborators 2003). Estimated absolute increases in breast cancer cases for estrogen-only reflect the effect of exposure time with 1.5–2 additional cases per 1,000 women with 5 years' use, as compared to five to six per 1,000 with 10 years use (Cancer 1997; Collaborators 2003). For users of combination HRT, results from the WHI (Rossouw et al. 2002) and the Million Woman Study (2003) suggests about six cases of breast cancer per 1,000 with 5 years of combination HRT use and 18–19 cases per 1,000 women with seven to 10 years of use. A systematic literature review suggests that the long-term use of combined hormones may be associated with higher mortality, later stage at diagnosis and a less favorable outcome (Collaborators 2003; Antoine et al. 2004).

In spite of significant debate regarding design limitations of the WHI and applicability to younger women, current recommendations for the use of combined and single-agent HRT are to manage the relief of moderate to severe menopausal symptoms on the basis of individual benefit and risk. The risks and benefits associated with alternative hormonal agents, doses, and time of exposure are currently unknown, although a summary of knowledge to date is provided at the conclusion of Chapter 17 (Ovarian Cancer Prevention). To aid the medical community in HRT use, a number of agencies and groups have published recommendations for HRT use for the treatment of menopause and associated bone loss. HRT is not recommended at present for the prevention of cardiovascular disease, dementia, or generally for long term use for disease prevention. Recommendations differ slightly by agency and by country, for US and non-US evidence-based treatment recommendations, we direct the reader to the National Guidelines Clearinghouse website (http://www.guideline.gov/). When prescribing HRT, a discussion of the most current evidence and an assessment of the potential benefit and harm should be provided to the individual patient. Because of the known risk of endometrial cancer for estrogen-only formulations, the US Food and Drug Administration (FDA) currently advises the use of combined (estrogen plus progesterone) HRT for the management of menopausal symptoms in women with an intact uterus at the lowest effective dose for the shortest time needed to abate symptoms (Palomares et al. 2006).

There are currently no formal guidelines for the use of HRT in women at high risk of breast cancer. Estrogens are contraindicated in women with a prior history of invasive disease, history of premalignancies or strong family history of breast cancer. This recommendation poses a significant challenge when confronted with a patient suffering severe menopausal symptoms. However, the reader is referred to Chapter 17 (Ovarian Cancer Prevention) of this text for a more in depth discussion of non-hormonal alternatives to treat the symptoms of menopause. Only a few studies have evaluated the effect of HRT after a diagnosis of breast cancer. The largest of these, the HABITS (hormonal replacement therapy after breast cancer – is it safe?) study was stopped early due to unacceptable

breast cancer recurrence and contralateral disease with 2 years' HRT use (hazard ratio of 3.5, 95% CI: 1.5–8.1) (Holmberg and Anderson 2004). In another randomized clinical trial (Loprinzi et al. 1994), no increase in risk of breast cancer recurrences were observed in women with a median follow up of 4.1 years. Use of progesterone-containing HRT was limited by intermittent use with continuous exposure avoided (Loprinzi et al. 1994). Though argued to be biased towards a high prevalence of cases with estrogen responsive tumors and greater use of formulations including progesterone, the HABITS study cautions against the use of hormonal agents in women with a history of breast cancer and others at increased risk.

In women at increased risk, including women with a history of breast cancer and those where estrogen exposure is actively suppressed with oophorectomy, aromatase inhibitors or SERMs, treating physicians may wish to rely on non-hormonal treatment strategies for the management of hot flashes, night sweats, and vaginal dryness (Mom et al. 2006; Cheema, Coomarasamy et al. 2007). There are no randomized clinical trials among women at increased risk of breast cancer or among women with a history of breast cancer that have assessed the overall efficacy or risks associated with many new treatments (e.g. clonidine, venlafaxine, gabapentin, and combination venlafaxine plus gabapentin) (Bordeleau et al. 2007). Use of these agents is controversial and should target severity of menopausal symptoms. Other hormone-based approaches, such as the use of low dose vaginal or dermal estrogens, is controversial in this population as most have been documented to raise circulating estradiol levels at least transiently, depending on the dose administered. There is less evidence to support the benefit of commonly used dietary isoflavones, black cohosh, or vitamin E. Non-hormonal alternatives to treat the symptoms of menopause are summarized in more detail in Chapter 17.

## 14.5 Prior Breast Health History

A history of breast cancer is associated with a three to four-fold increased risk of a second primary cancer in the contralateral breast (Page and Jensen 1994; Kollias et al. 1999; Page et al. 2003). Presence of any premalignant ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) confers an eight to 10-fold increase in risk of developing breast cancer in women who harbor untreated preinvasive lesions (Page 2004; Ashbeck et al. 2007). The introduction of widespread screening has resulted in a nearly 5-fold increase in the detection of in situ or locally-contained disease (i.e., cancerous type lesions of the ducts and terminal lobular units that have not breached the basement membrane). The majority of these early-stage lesions are diagnosed as DCIS, which are often not clinically appreciated and only detectable by imaging methods (Ernster et al. 1996). Nearly 55,000 women or 20% of all breast lesions diagnosed annually in the US are DCIS. The increase incidence rates of DCIS have occurred in all age groups but especially among women over age 50. Because of the high risk of invasive disease in women with DCIS, surgical excision followed by radiation therapy and long-term use of tamoxifen for ER positive lesions has been widely adopted as treatment to prevent

invasive disease. Among women in whom tamoxifen use is contraindicated, aromatase inhibitors are now being extensively used despite absence of strong clinical trial data. The current NSABP trial comparing tamoxifen to aromatase inhibitors in women with DCIS are complete but results are pending publication.

A second, less common type of in situ disease, LCIS, accounts for approximately 15% of in situ lesions. The incidence rate of LCIS is increasing at about two times the rate of invasive cancers, particularly among postmenopausal women (Li et al. 2002). In LCIS, atypical cells occur throughout the breast lobules with both breast involved in approximately one-third of patients. LCIS often involves the entire breast parenchyma and is characterized by multifocality and a high rate of bilaterality (30–50% of cases). Mammograms of women with LCIS show sheet-like areas of breast density with higher percentages of fibroglandular density compared to age-matched controls. LCIS confers an increased risk of invasive breast cancer in either breast with cancer arising in either ducts or lobules. Though controversial and difficult to diagnose given the multifocal and bilateral nature of the lesions, LCIS confers between a 6 and 12-fold increase in risk for the development of invasive carcinoma (Simpson et al. 2003). Crisi and colleagues concluded that LCIS on core biopsy is a significant marker for concurrent and near-term breast pathology requiring intensive clinical follow up with specific individualization of patient care (Crisi et al. 2003). Current recommendations support more active surveillance in women with these lesions. Adjuvant use of tamoxifen in women diagnosed with these high-risk premalignant lesions has proven efficacious in both LCIS and DCIS patients for the prevention of invasive cancers. Tamoxifen for 5 years has been shown to cut the risk of ER positive invasive cancers by half in women with a history of LCIS or DCIS, an effect that appears to be sustained for at least 10 years (Fisher et al. 1998; Cuzick et al. 2007). At present, patients should understand that there are no data that support a survival advantage for women who choose tamoxifen to prevent the development of invasive disease. Longer follow-up studies are needed to determine the long-term benefit of tamoxifen in terms of survival. At present, there are limited options for women with ER negative DCIS other than surgical removal and radiation therapy. It should be noted that approximately 25% of high grade DCIS (i.e., high-risk lesions) lack ER expression and share features with associated invasive disease in the same individual (Steinman et al. 2007). With advances in molecular profiling and improved detection of small lesions that harbor aggressive features, clinical treatment of such lesions may soon mirror the differential treatment of early stage tumors where molecular subtype characterization overrides the importance of tumor size and other previously used prognostic indicators.

A history of breast biopsy that is positive for hyperplasia, fibroadenoma with complex features, sclerosing adenosis and solitary papilloma have been associated with a modest 1.5- to 2-fold increase in risk of breast cancer (Page 2004; Ashbeck et al. 2007). In contrast, any diagnosis of atypical hyperplasia that is ductal or lobular in nature, especially if diagnosed under the age of 45 years, carries a four to five-fold increased risk of breast cancer with risk rising to a eight to 10-fold risk among women with mulitple foci of atypia or calcifications in the breast (Degnim et al. 2007). Benign pathologies of the breast, including fibrocystic disease such as fibrocystic change without proliferative breast disease or fibroadenoma, have not been associated with increased risk (Dupont et al. 1994).

## 14.6 Lifestyle Risk Factors

The wide range of breast cancer incidence rates around the world is largely attributed to differences in dietary intake and reproductive behaviors (Henderson and Bernstein 1991; Kaaks 1996; Stoll 1998a,b; Holmes and Willett 2004). As with cancers of the colon and prostate, diets that are enriched in grains, fruits and vegetables, low in saturated fats, and low in energy (calories) and alcohol are thought to be protective against breast cancer (Holmes and Willett 2004). Postmenopausal breast cancer is positively associated with adult weight gain, a Western diet (high energy content in the form of animal fats and refined carbohydrates), and a sedentary lifestyle. The Western lifestyle (e.g., Western diet and lack of exercise) strongly correlates with the development of obesity, particularly abdominal obesity, and chronic states of hyperinsulinemia and higher production and availability of insulin like growth factor (IGF)-1 and endogenous sex hormones through suppression of sex hormone binding globulin (Kaaks 2004; Lukanova et al. 2004). Although dietary fat has not emerged as a consistent risk factor for breast cancer, evidence implicating high fat, high energy diets in the shift towards earlier onset of menses in young women in Western cultures, if corroborated, may serve as the basis for future recommendations regarding lifetime breast cancer risk reduction starting with diet modification in childhood (Law 2000). Similar recommendations for reduced sugar intake may also follow with accumulating evidence to modify risks mediated through insulin and IGF hormones. The role of diet, physical activity and body composition in cancer prevention is covered extensively in Chapter 3, with an emphasis on studies related to breast cancer. We refer the reader to this chapter and also recommend the comprehensive Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective published jointly by the World Cancer Research Fund and the American Institute for Cancer Research (AICR, 2007).

## 14.7 Risk Factors and Tumor Subtypes

In 1995, Potter and colleagues reported an inverse association between parity and $ER^+/PR^+$ tumors that was not present for $ER^-/PR^-$ tumors (Potter et al. 1995). A number of smaller studies have investigated the relationship between established breast cancer risk factors and hormone receptor status. This body of work concluded that there were no major differences in risk factors by hormone receptor status or in tumor etiology by receptor status (Stanford et al. 1987; Cooper et al. 1989; Habel and Stanford 1993). More recently, the Nurses' Health Study found that parity and early age of first birth were inversely associated with $ER^+/PR^+$ tumors but not with $ER^-/PR^-$ tumors (Colditz et al. 2004). These data were replicated in a meta-analysis (Ma et al. 2006a,b). More recently, a limited number of studies using tumor markers to subclassify tumors into the newly defined five tumor classes support the evidence favoring the presence of distinct reproductive risk factor profiles among tumor subtypes (Millikan et al. 2007; Yang et al. 2007). The work by Millikan and colleagues (Millikan et al. 2007) found that luminal A tumors (strongly $ER^+$) showed the

expected association with reproductive risk factors. However, the basal-like tumors (so-called triple negatives) appeared to be positively associated with high parity (particularly among women with short or failed breastfeeding), young age at first full-term pregnancy, elevated early waist to hip ratio (or abdominal obesity), and weight gain in childhood, as well as with earlier age at menarche (Millikan et al. 2007). These findings were largely consistent with that of Yang and colleagues (Yang et al. 2007). It is notable that whereas these risk factors (i.e., high parity, early age of menarche) occur more commonly in African American women and are positively associated with lower socioeconomic status, the positive association with risk was present for both White women and African American women and independent of menopausal status. These data support the growing consensus that breast cancers are not homogeneous, but rather a group of separate diseases with distinct origins. Looking forward, this suggests that risk assessment tools will need to stratify individuals for disease subtypes. Future tumor type studies are needed to fully clarify the importance of reproductive behaviours, hormone use, diet and environmental factors on individual risk of specific tumor types.

## 14.8 Emerging Breast Cancer Risk Factors for Patient Management

A panel of experts on breast cancer risk factors comprising the Breast Cancer Prevention Collaborative (BCPC) has cited the quantitative measure of breast density as a high priority risk factor for validation with the specific intent to more efficaciously deliver prevention agents. There is convincing evidence that extensive areas of radiographically-dense tissue in the breast are an independent risk factor for the development of breast cancer (Saftlas et al. 1991; Boyd et al. 1995; Salminen et al. 2000; Byrne et al. 2001; Nieminen et al. 2003; Stines and Tristant 2005). The association between breast density and cancer risk is present using both quantitative (Boyd et al. 1995; Byrne et al. 1995; Boyd et al. 1998; Prevrhal et al. 2002; Vacek and Geller 2004) and qualitative (Saftlas et al. 1989; Wolfe et al. 1987; Prevrhal et al. 2002; Ziv et al. 2003; Vacek and Geller 2004; Ziv et al. 2004; Tice et al. 2005) measures of the breast image obtained by standard mammography. The presence of dense tissue that occupies more than 50% of the area of a mammographic image, which occurs in up to 30% of postmenopausal women, is associated with a three to 5-fold elevated risk of breast cancer (Wolfe et al. 1987; Saftlas et al. 1989; Boyd et al. 1995; Byrne et al. 1995; Boyd et al. 1998). Several groups have found the addition of mammographic density to current risk assessment models modestly improves the relative and absolute prediction of risk (Barlow et al. 2006; Chen et al. 2006; McCormack and dos Santos Silva 2006; Palomares et al. 2006). A recent article by Kerlikowske and colleagues shows that breast cancer risk estimation can be improved at an individual level by including at least two longitudinal measures of Breast Imaging-Reporting and Data System (BI-RADS) breast density scores (Kerlikowske et al. 2007a). The implementation of BI-RADS measurement would be largely straightforward from a clinical perspective. In the Kerlikowske study, women who experienced an increase in their BI-RADS breast density category over an average of 3 years had a higher risk of developing breast cancer.

For example, among those women whose BI-RADS category increased from one to two, or from one to three, over a 3-year time period had a double or triple increase in risk, respectively, compared to women who remained unchanged from the initial BI-RADS category one. This increase in risk with each rise in category was evident only for women initially categorized with BI-RADS categories one through three. Women with an initial classification of BI-RADS category four suffered the highest adjusted rates of breast cancer and demonstrated no reduction in risk if their BI-RADS score decreased at follow up. This observation is consistent with earlier work by Boyd and colleagues that suggests that the elevated breast cancer risk associated with highly dense breasts persists up to 8–10 years following initial evaluation (Boyd et al. 2005). The availability of computer-aided systems to accurately quantify mammographic density is likely to bring routine assessment of a woman's mammographic density to the clinic in a relatively short time period.

## 14.9 Environmental Risk Factors

A number of environmental exposures, including tobacco smoke (both active and passive exposure), dietary (e.g. charred and processed meats) and environmental carcinogens, such as exposure to pesticides, irradiation and environmental and dietary estrogens, have been investigated in relation to breast cancer risk in humans (Laden and Hunter 1998; Calle et al. 2002; Coyle 2004; Gammon et al. 2004; Gammon et al. 2004). Of these environmental exposures, only high doses of ionizing irradiation to the chest area during puberty has been unequivocally linked with an increased risk of breast cancer in adulthood (Carmichael et al. 2003). Because of the strong association between ionizing irradiation and breast cancer risk, care is given in medical diagnostics procedures to minimize exposure to the chest area, particularly during adolescence. Women with a prior history of irradiation exposure to the chest area should be examined and counseled on their risk of breast cancer based upon timing and dose of prior exposure. A patient treated for Hodgkin's lymphoma, particularly at age 30 or earlier, has nearly a 5-fold increased risk of developing breast cancer. This risk increases to nearly 20-fold for women treated during their adolescence (Clemons et al. 2000). Current evidence does not support a significant and reproducible link between other environmental exposures and breast cancer risk. Thus, a number of factors remain suspect but unproven.

## 14.10 Screening and Early Detection

Early detection remains the primary defense available to patients to prevent the development of life-threatening breast cancer. Detection of breast tumors that are smaller or non-palpable and that present with a favorable tumor marker profile are more treatable when detected early and thus have a more favorable prognosis. The survival benefit of early detection with mammography screening has been demonstrated (Berry et al. 2005; Elmore

et al. 2005). Therefore, early detection is widely endorsed by organizations that issue clinical recommendations for breast cancer care. The most widely recommended method in the US is annual screening mammography beginning at age 40 years. For women younger than 40 years of age, monthly breast self-exam practices and clinical breast exams every 3 years are recommended, beginning at 20 years of age (Smith et al. 2003).

## 14.11 Breast Self Exam (BSE) and Clinical Breast Exam (CBE)

Both BSE and CBE involve inexpensive and noninvasive procedures for the regular examination of breasts (i.e., monthly for BSE and annually for CBE). Evidence supporting the effectiveness of BSE and CBE are controversial and largely inferred. Even with appropriate training, BSE has not been found to reduce breast cancer mortality (Thomas et al. Cancer 1997). However, with increasing improvements in treatment regimens for early, localized disease, BSE and CBE, particularly among women younger than 40 who otherwise have no screening options, are still considered promising and safe methods of intervening early and continue to be recommended (Vahabi 2003). Most recently, randomized clinical trial results support combining CBE with mammography to enhance screening sensitivity, particularly in younger women where mammography may be less effective and in women receiving mammograms every other year as opposed to annually (Shen and Parmigiani 2005).

## 14.12 Mammography

Mammography has been demonstrated as an effective tool for the prevention of advanced breast cancer in women at average risk. Mammography is currently the best available population-based method to detect breast cancer at an early stage when treatment is most effective (Nystrom et al. 2002; Tabar et al. 2003; Elmore et al. 2005; Swedish Organised Service Screening Evaluation Group 2006). Mammography often detects a lesion before it is palpable by CBE and, on average, 1–2 years before noted by BSE. The US Preventive Services Task Force estimates the benefit of mammography in women between 50 and 74 years of age to be a 30% reduction in risk of death from breast cancer. For women age 40–49, the risk of death is decreased by 17% (Humphrey et al. 2002). In 2005, Berry and colleagues concluded that screening and treatment both contribute to the recent declines in deaths from breast cancer; furthermore, the decline is explained by accounting for screening and therapy that is most effective for earlier stage disease (Berry et al. 2005). Although mammography guidelines have been in place for over 30 years, between 20 and 30% of women still do not undergo screening as indicated. The two most significant factors for a woman to undergo mammography are physician recommendation and access to health insurance. Non-White women and those of lower socioeconomic status

remain less likely to obtain mammography services and more likely to present with life-threatening, advanced stage disease (Ward et al. 2004).

## 14.13 Alternative Screening Modalities and Future Directions

While mammography remains the most cost effective approach for breast cancer screening, the sensitivity (67.8%) and specificity (75%) are not ideal (Berg et al. 2004). As reported (Vahabi 2003), mammography combined with CBE slightly improves sensitivity (77.4%) with a modest reduction in specificity (72%) (Berg et al. 2004). Comparisons between recently introduced digital mammography and screen-film mammography suggest that the sensitivity of full-field digital mammography is superior to screen film mammography in certain subsets of women (Brem et al. 2003; Del Turco et al. 2007). For example, digital mammography demonstrates improved detection rates for younger women and for women with more dense breasts. Improved imaging modalities with greater sensitivity are of particular benefit for women at the highest risk and for women whose breast images are difficult to interpret. Ultrasound has become a widely available and useful adjunct to mammography in the clinical setting. Ultrasound is generally used to assist the clinical exam of a suspicious lesion detected by mammogram or physical examination. As a screening device, the ultrasound is limited by a number of factors but most notably by the failure to detect microcalcifications and poor specificity (34%) (Smith et al. 2003; Berg et al. 2004).

In an effort to overcome the limitations of mammography and ultrasound, magnetic resonance imaging (MRI) has been explored as a modality for detecting breast cancer in women at high risk and in younger women. A combination of T-1, T-2 and 3-D contrast enhanced MRI techniques has been found to be highly sensitive (approximating 99% when combined with mammogram and clinical breast exam) to malignant changes in the breast (Liberman et al. 2003; Morris et al. 2003; Liberman 2004; Berg et al. 2004). MRI has been demonstrated to be an important adjunct screening tool for women with *BRCA1* or *BRCA2* mutations (Kriege et al. 2004), identifying cancers at earlier stages (Warner et al. 2004). However, breast MRI has limited utility as a general screening tool with a 10-fold higher cost than mammography and poor specificity (26%) resulting in significantly more false positive reads that generates significant additional diagnostic costs and procedures (Berg et al. 2004; Kriege et al. 2004). In the most recent American Cancer Society (ACS) Guidelines for Breast Screening with MRI as an Adjunct to Mammography (Saslow et al. 2007), women at highest risk (e.g. those with an approximately 20–25% or greater lifetime risk of breast cancer), such as *BRCA1/2* mutation carriers, women with a strong family history of breast or ovarian cancer and women who received irradiation for the treatment of Hodgkin disease, are recommended to receive breast MRI with annual mammogram as active surveillance for early cancer detection starting at age 30 years (Saslow et al. 2007). The ACS does not recommend the use of MRI in women at average risk of breast cancer. Among those at average risk, a combination of CBE and yearly mammograms is recommended. There is not sufficient evidence to support the use of MRI in women with

a personal history of breast cancer or for women with a prior history of LCIS or atypical hyperplasia. The ACS concluded that the use of MRI "should be decided on a case-by-case basis, based on factors such as age, family history, characteristics of the biopsy sample, breast density, and patient preference (Saslow et al. 2007)."

## 14.14 Primary Prevention of Breast Cancer

Pharmacologic and surgical strategies have demonstrated efficacy in lowering the incidence of breast cancer in women at high risk (Smith et al. 2003; Newman and Vogel 2007; Vogel 2007). However, most breast cancer cases do not arise in a readily identifiable group of women at known risk but occur sporadically in the general population. At present, there are no practice guidelines for the prevention of breast cancer for women with average to moderate breast cancer risk. It is recommended that women maintain a healthy body weight throughout their adult life, engage in regular physical activity, breastfeed, eat diets rich in grains, fruits and vegetables, minimize exposure to exogenous hormones and consume alcoholic beverages only in moderation, if at all (Byers et al. 2002; Cerhan et al. 2004; Key et al. 2004). The ACS provides a set of recommendations on their web site, which can be accessed at www.cancer.gov/cancertopics/pdq/prevention/breast.

Future recommendations for the general population are likely to include a greater emphasis on the reduction of simple sugars in the diet and decreasing abdominal obesity. Further research on the influence of dietary fat on reproductive risk factors, such as age of onset of menarche, is needed. These findings will likely guide future recommendations that may shift the delivery of prevention counseling related to lifestyle factors from adults to children and their parents. It is likely that earlier lifestyle modification will have the greatest impact on lifetime risk reduction.

There are several options for the primary prevention of breast cancer in women at significantly increased risk (e.g. *BRCA1/2* mutation carriers, women with multiple affected relative with breast or ovarian cancer presenting at early age, and women with a personal history of breast cancer or history of premalignant disease such as DCIS or LCIS). Available options are aimed at removing tissue before it can become cancerous and at decreasing endogenous hormone exposure. Current prevention strategies include intervention with bilateral mastectomy, salpingo-oophorectomy or the use of selective ER modulators (e.g., SERMs) (Newman and Vogel 2007).

Bilateral prophylactic mastectomy and bilateral salpingo-oophorectomy are considered highly effective options for risk reduction in women at very high risk. Bilateral prophylactic mastectomy (BPM) has been associated with as much as an 85% reduction in breast cancer risk among *BRCA1* and *BRCA2* mutation carriers (Hartmann et al. 1999; Hartmann et al. 2001; Meijers-Heijboer et al. 2001; Rebbeck et al. 2004). While BPM is most effective in lowering breast cancer risk, bilateral salpingo-oophorectomy (BSO) (Morris et al. 2003) is currently the most common choice for *BRCA* mutation carriers in the US and Canada (Narod and Foulkes 2004; Rubinstein 2005). BSO alone followed by short

exposure to HRT is associated with a 90% reduction in ovarian cancer risk and a 50% or more lowering of breast cancer risk (Rebbeck et al. 1999, 2002, 2005). Risk reduction is greatest when BSO is performed early during the premenopausal years (Kramer et al. 2005). The current recommendation is that *BRCA1/2* mutation carriers should consider undergoing BSO by age 35–40 years or at the time that childbearing is completed. Materials are available to aid with patient decision making to undergo BPM or BSO. One valuable resourse is a text entitled "Ovarian Cancer: Risk-Reducing Surgery," which may be requested from surgerybook@fccc.edu or by calling the Margaret Dyson Family Risk Assessment Program at 1–800–325–4145. It is very important that patients take the time to give appropriate thought and consideration to this major decision and that the patient is aware of the risks and benefits associated with these and other options to reduce risk before deciding to undergo BPM or BSO.

It is important to recognize that only a portion of women at very high risk and even fewer women at moderate risk will ultimately develop breast cancers. Thus, a great benefit to the individual patient will be gained with improvements in the accuracy of risk assessment. For example, as we gain more information on the types and effects of specific mutations in the *BRCA* genes, identify and validate new gene determinants and understand the role of modifying genes and the effects of the environment, we will see improved stratification of women into risk categories and targeted chemoprevention therapies. This will reduce the need for many women to unnecessarily undergo aggressive surgical procedures.

## 14.15 Chemoprevention

The use of natural or synthetic chemical agents to prevent, reverse or suppress carcinogenic events in the breast defines the practice of breast cancer chemoprevention. This definition of chemoprevention excludes the use of dietary (e.g. whole foods), lifestyle or behavioral interventions. The goal of chemoprevention is to reduce the incidence of breast cancer by inhibiting or delaying the progression of premalignant mammary epithelial cells. Thus, well-designed randomized clinical trials that demonstrate agent efficacy and define an appropriate risk to benefit ratio are prerequisite to improving the clinical practice of breast cancer prevention. Several key chemoprevention agents for the reduction of breast cancer risk are discussed in more detail below.

### 14.15.1 Selective Estrogen Receptor Modulators (SERMs)

Having identified estrogen exposure as a risk factor for breast cancer, it was not surprising that adjuvant treatment trials with the selective ER modulator, tamoxifen, showed not only reduced recurrence of breast cancer among women with breast cancers, but also a reduction in the number of second primary breast cancers (Nayfield, Karp, et al., 1991). Based on these

data, a large randomized clinical trial with tamoxifen was initiated by the National Surgical Adjuvant Breast and Bowel Project (NSABP) to test its efficacy as a chemoprevention agent among women at increased risk of breast cancer. There was a 49 percent reduction in the incidence of invasive breast cancer and a 50 percent reduction in DCIS among patients randomized to tamoxifen compared to those receiving placebo. The benefit was exclusive for lowering the risk of ER-positive disease, consistent with the known mechanism of action. The greatest benefit was achieved among women with a prior history of LCIS (56%) or atypical hyperplasia (86%). In addition, it was noted that fewer fractures occurred among those treated with tamoxifen, suggesting a added protective effect on the bone. However, a greater number of endometrial cancers, pulmonary embolism, strokes and deep vein thromboses were observed among women taking tamoxifen (Fisher, Costantino, et al., 1998). Subsequent analyses demonstrated that the overall health benefit for the patient depended on individual risk of breast cancer, endometrial cancer, and thrombosis (Gail, Costantino, et al., 1999). In 2002, an analysis of the current evidence by the US Preventive Services Task Force (USPSTF) resulted in the recommendation for the routine use of tamoxifen or raloxifene for the primary prevention of breast cancer only for women at high risk for breast cancer. The USPSTF cautioned about the greater incidence of adverse events in older women, suggesting an improved risk-to-benefit ratio profile for younger women (Kinsinger, Harris, et al., 2002). The summary evaluation of the task force resulted in recommendations to clinicians to discuss the benefits and risks of these interventions with patients at high risk of breast cancer.

To further improve efficacy, additional SERMs with lower toxicity, such as raloxifene, have been developed. Within the context of a secondary analysis of the Multiple Outcome of Raloxifene Evaluation study (MORE Study), postmenopausal women randomized to raloxifene had a 72 percent reduction in breast cancer incidence compared to women randomized to placebo (Cauley, Norton, et al., 2001). Greatly encouraged by these data, NSABP investigators conducted a study to compare the effectiveness of raloxifene to tamoxifen in a randomized, double-blind study of postmenopausal women at high-risk of breast cancer (The STAR Trial). In 2006, the STAR Trial reported near equal incidence of invasive breast cancers between tamoxifen and raloxifene, but fewer thromboses and cataracts in women on raloxifene compared to tamoxifen (Vogel, Costantino, et al., 2006). The incidence of strokes, fractures and cardiovascular disease did not differ between groups. Consistent with a previous study (Martino, Cauley, et al., 2004), the incidence of premalignant DCIS and LCIS were statistically higher among those receiving raloxifene compared to the tamoxifen, suggesting no or little activity for raloxifene in these lesions. A summary of management options for patients at high risk of breast cancer is presented in Table 14.3, and a summary of general recommendations for all women is presented in Table 14.4.

### 14.15.2 Aromatase Inhibitors

Aromatase inhibitors represent a newer class of agents targeting estrogen production at the tissue level by specifically inhibiting the last step in estrogen biosynthesis. There are two general classes of aromatase inhibitors: irreversible steroidal activators and reversible nonsteroidal imidazole-based inhibitors. Aromatase inhibitors act to suppress the

**Table 14.3** Current patient management options for women at high risk for breast cancer: benefits and risks

| | Surveillance | Bilateral Oophorectomy | Risk reduction mastechtomy | Tamoxifen/aromatase inhibitor[a] |
|---|---|---|---|---|
| Benefits | Non-invasive, non-toxic | Significant lowering of risk among premenopausal women | Significant risk reduction (>90%) in all high risk women including BRCA1/2 carriers | Approximate 50% reduction in ER (+) tumors |
| | Promise of new methods with improved sensitivity (e.g., MRI) | Risk reduction observed in both BRCA1/2 carriers, greatest among BRCA2 carriers undergoing surgery before 35 years | | Greatest benefit for women with history of premalignant disease or family history ('high risk' women) |
| | | | | Effective against only ER (+) disease |
| | | | | Limited data suggest efficacy in BRCA carriers |
| Risks | Lack of sensitivity in young women | Premature menopause | Extreme | Increased risk of thrombotic and endometrial cancer events (TAM Only) |
| | Concerns over low dose irradiation exposure | Irreversible | Psychological | |
| | Lack of strong evidence that early detection reduces mortality for all women, particularly in *BRCA1/2* carriers | Psychological/ quality of life | Irreversible | No efficacy for ER (-) tumors (TAM & AI) |
| | | | | Efficacy in BRCA carriers not established |
| | | | | Age of initiation and duration for optimum health benefit unknown |
| | | | | Overall health benefit not demonstrated |

[a]Under investigation for use in primary prevention

**Table 14.4** Recommendations for all women to lower the lifetime risk of breast cancer

| |
|---|
| • Breastfeeding[a] is encouraged with each child |
| • Maintain healthy body weight appropriate to height throughout adulthood |
| • Assuming normal body weight at age 18, maintain adult body weight within 5–10% |
| • Engage in regular physical activity throughout life (such as 10,000 steps of walking at a vigorous pace 4–5 times per week) |
| • Eat a diet rich in fruits, vegetables and grains |
| • Eat a diet low in total fats and refined sugars |
| • Substitute saturated fats in diet with unsaturated healthy fats |
| • Drink alcoholic beverages in moderation |
| • Weigh risk and benefits of hormone replacement therapy |
| • Use HRT for shortest interval to manage menopausal symptoms |
| • Follow recommended screening guidelines |
| • Practice monthly self breast exam |
| • Schedule and attend regular health checkups |

[a]While the protective effect of breastfeeding is better established, there are currently no established guidelines on the necessary length to achieve benefit

low levels of estrogen production in postmenopausal women but are largely ineffective in women whose estrogen production is primarily from their ovaries (e.g. premenopausal women) (Jordan and Brodie 2007).

Clinical trials of aromatase inhibitors suggest that aromatase inhibitors may be effective in the primary prevention of breast cancer in the postmenopausal setting. Results from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial and Breast International Group (BIG) 1–98 trial suggest that aromatase inhibitors are better at reducing recurrence and contralateral breast cancers as well as not having the toxicity profile that is associated with tamoxifen use (i.e., endometrial cancer, venous thromboembolism) (Glass et al., 2007; Breast International Group 1–98 Collaborative, et al. 2005). The failure to achieve a survival benefit with tamoxifen in the prevention setting has challenged its use in primary prevention. Superior reductions in contralateral breast cancers and improved disease free survival with fewer serious adverse events has led to widespread interest in evaluating the efficacy of aromatase inhibitors for the primary prevention of invasive breast cancer for postmenopausal women at high risk of breast cancer. However, in contrast to the SERMS, aromatase inhibitors carry significant risk for bone health, which may limit their use in the prevention setting. To assess efficacy in the primary prevention setting, two large clinical trials are currently being conducted to assess the role of aromatase inhibitors to prevent postmenopausal breast cancer. The International Breast Cancer Intervention Study II (IBIS II) began enrollment in 2003. IBIS II is designed to compare 5 years of daily anastrozole to placebo in 4,000 postmenopausal women at high risk of breast cancer (Cuzick 2008). The National Cancer Institute of Canada Clinical Trials Group began enrollment to the ExCel Breast Cancer Prevention Trial, which compares exemestane to placebo among postmenopausal women at high risk of breast cancer (Goss et al. 2007).

### 14.15.3 Retinoids

Vitamin A analogs or retinoids have been shown to inhibit the in vitro and in vivo growth of breast tumor cells (Serrano et al. 2004). Two types of nuclear retinoid receptors, the retinoid X receptor (RXR) and the retinoic acid receptor, bind to the retinoids to mediate their transcriptional effects on genes involved in controlling cell proliferation, differentiation and apoptosis. The retinoids or vitamin A analogs have shown promise as agents for primary breast cancer prevention; however, issues related to their toxicity pose a challenge for dosing and ultimate efficacy. There is promise in the development of less toxic synthetic retinoids, such as *N*-4-hydroxyphenyl retinamide (4-HPR, fenretinide) and specific modulators of RXR that prevent tumor development in chemically-induced animal models of mammary tumorigenesis, including ER-negative tumors in mouse models. Results from an early randomized breast cancer trial (Decensi et al. 2003) evaluating the efficacy of fenretinide to prevent a second breast malignancy in women with cancer were disappointing, with no overall reduction in risk observed. Secondary analyses have raised questions about the interaction of retinoids on the IGF system and potential effects of age and menopausal status. Animal studies have suggested a potential benefit of the retinoids for the less common, but more aggressive, ER-negative tumors. Ongoing studies to identify intermediate response biomarkers for these agents may prove informative for the design of future retinoid-based prevention trials (Zanardi et al. 2006).

### 14.15.4 Non-Steroidal Anti-Inflammatory Drugs (Nsaids)

Several large epidemiological studies provide strong and compelling evidence for a protective role of NSAIDs to reduce the risk of breast cancer (Johnson et al. 2002; Davies 2003; Harris et al. 2003). In a study of 80,741 postmenopausal women participating in the prospective Women's Health Initiative (WHI) Observational Study, regular NSAID use (largely restricted to ibuprofen or aspirin) of two or more tablets per week was associated with a 21% lower incidence with 5–9 years of use and a 28% reduced incidence with 10 years of use (Harris et al. 2003). There was a statistically significant inverse linear trend of breast cancer incidence with the duration of use. More recent studies report a 20–30% reduced risk in breast cancer incidence with NSAID use (Gill et al. 2007). The most recent evidence suggests the risk reduction is limited to ER-positive disease (Terry et al. 2004; Gill et al. 2007). These studies highlight the need for clinical trials to determine the efficacy of NSAIDs as cancer prevention agents and, ultimately, to determine their specificity for disease subtypes (e.g., ER-positive versus ER-negative).

The use of NSAIDs is not a novel concept for chemoprevention research. However, prevention trials evaluating the cyclooxygenase type 2 (COX-2) specific NSAIDs (e.g. celecoxib, rofecoxib, and valdecoxib) are more recent developments in chemoprevention research. Interest in COX-2 specific NSAIDs has been spurred by their improved toxicity profile as compared to other NSAIDs (e.g. reduced gastrointestinal toxicity) and proven efficacy in preventing colorectal adenomas (Bertagnolli 2007a,b). The emergence of worrisome cardiotoxicity associated with the twice daily dosing of COX-2 inhibitors has

led to the closure of many trials and reevaluation of risk and application of COX-2 inhibitors in the prevention setting for breast cancer despite the strongly positive risk reductions seen in clinical trials and epidemiologic studies (Harris et al. 2007). COX-2 inhibitors remain under investigation for the chemoprevention of other cancers (e.g., cervical cancer chemoprevention trials are underway using celecoxib). Additional effort is needed to evaluate the effect of aspirin and NSAIDs with lower toxicity for the prevention of breast cancer.

### 14.15.5 Other Agents

There are a number of additional compounds that do not act through the modulation of estrogen or the estrogen receptor that have demonstrated activity in animal models of breast and other solid tumors. Interest in these agents is strong because of the potential for the broader, ER-independent action of these agents. In addition, agents that target the retinoid receptor or the use of NSAIDs, tyrosine kinase inhibitors, antibodies that target the epidermal growth factor receptor (EGFR) signaling pathway, specific inhibitors of COX-2, inducers of apoptosis, modulators of the IGF signaling pathways and inhibitors of angiogenesis are under investigation as chemoprevention agents for breast cancer (Shen and Brown 2003; Uray and Brown 2006). Most of these agents are in preclinical or early phase development for use in breast cancer. Clinical trial data for these agents are likely to emerge by 2010.

## 14.16 Risk Assessment and Clinical Applications

One of the primary challenges to successful prevention in the breast is the need for accurate assessment and communication of risk to the individual patient or research subject for prevention trials (refer to Chapter 5 for a more thorough discussion of the role of genetic counseling in the hereditary risk of cancer). The development of clinical practice guidelines that allow for informed decision making regarding surveillance and the weighing of benefits and risks of available prevention options remains hindered by the lack of accuracy in determining risk at the level of the individual patient. Risk assessment practice guidelines are likely to continue to improve as more information on risk factors, molecular biology of premalignancies, and their predictive potential are incorporated into existing models.

### 14.16.1 Elevated Risk

Elevated risk of breast cancer has been defined across groups as the presence of any factor that is reliably identifiable (i.e., family history, prior history of atypia) and when present has been consistently associated with an increase in risk that nears twice that or greater of the general population. In general, a low elevation in risk has been associated with factors that confer 1.5- to 2-fold increase over the general population of women at average risk.

Moderate risk is associated with a three to five-fold increase in risk, and high risk is associated with factors that confer a greater than five-fold elevated risk. Patients with moderate or high risk of breast cancer should be counseled on the importance of early detection and surveillance methods and should be informed on the benefits and risks of primary prevention and chemoprevention.

### 14.16.2 Risk Assessment Models

There has been a concerted effort by several groups to develop multivariate methods to derive a Breast Cancer Risk Assessment Tool (http://www.cancer.gov/bcrisktool/) using sets of risk factors (genetic and other) that are informative for estimating the risk of breast cancer. Two types of risk models have been developed that are clinically relevant – those that estimate a woman's absolute risk of developing breast cancer over time and those that determine the likelihood that an individual is a carrier of a *BRCA1* or *BRCA2* or unknown gene mutation (i.e., *BRCA1/2* Probability Models) (Nelson et al. 2005; Culver et al. 2006). The models that predict mutation carrier status and their clinical use have been discussed briefly above under genetic risk factors. A review of these models and their performance relative to each other is available elsewere (Nelson et al. 2005) (Culver et al. 2006).

The US Preventive Services Task Force (USPSTF) has assigned a B recommendation, indicating that there is fair evidence that women with sufficient family history suggestive of deleterious mutations in *BRCA1* or *BRCA2* genes be referred for genetic counseling and evaluation for BRCA testing. Several risk tools have been developed to aid the physician and patient in the decision to seek further genetic testing for hereditary breast cancer (Culver et al., 2006). These include a model developed by Myriad Genetic Laboratories, the Couch model, BRCAPRO (most widely used), the Tyrer–Cuzick model and the more recently developed Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) (Culver et al. 2006). The USPSTF does not specifically endorse any of these genetic risk assessment models because of insufficient data to evaluate their applicability to asymptomatic, cancer-free women. The USPSTF does support the use of a greater than 10% risk probability for recommending further evaluation with an experienced genetics counselor for decisions regarding genetic testing.

In contrast to BRCA probability tools, risk prediction models are designed to derive individual risk estimates for the development of breast cancer over time. These models are presented in Table 14.5 and include the Claus (or CASH) Model, the Tyrer–Cuzick Model, and the Gail Model. The Gail Model was originally developed in 1989 from data derived from the Breast Cancer Detection and Demonstration Project (BCDDP) (Gail et al. 1989). It was developed to estimate the probability of developing breast cancer over a defined age interval and was originally intended to improve screening guidelines. The model was subsequently revised (Gail Model 2) and validated to predict risk of invasive breast cancer including information on history on first-degree affected family members (Costantino et al. 1999; Rockhill et al. 2001). The Gail Model 2 has been used extensively in clinical practice and has served as the basis of eligibility for a number of the breast cancer prevention trials (Fisher et al. 1998). At present, the US FDA guidelines utilize the NSABP's

**Table 14.5** Comparison of risk factors used in risk assessment models in women with no prior history of invasive breast cancer or carcinoma in situ

| Model | Gail | Claus/Ford[a] | Tyrer–Cuzick/manual |
|---|---|---|---|
| | Age<br>Reproductive<br>Age menarche<br>Age first live birth | Age<br>Reproductive<br>None | Age<br>Reproductive<br>Age menarche<br>Age menopause<br>Age first live birth |
| | Personal history<br>biopsy<br>ADH[b] | Personal history<br>None | Personal history<br>Biopsy<br>Atypical hyperplasia<br>LCIS |
| | Family history<br>First degree relative | Family history<br>First degree relative<br>Second degree relative<br>Age of onset<br>Ovarian cancer (Ford)<br>Male breast cancer (Ford) | Family history<br>First degree relative<br>Second degree relative<br>Age of onset<br>Ovarian cancer<br>Male breast cancer (Manual) |
| | Lifestyle<br>None | Lifestyle<br>None | Lifestyle<br>BMI[c] (Tyrer–Cuzick) |

[a]Output BRCAPro software package
[b]Atypical ductal hyperplasia
[c]Body mass index as a surrogate for adult weight

modified Gail model as the basis for eligibility for the prophylactic use of tamoxifen. Tamoxifen is approved for women 35 years and older who have a 5-year Gail risk of breast cancer of 1.67 or greater (Freedman et al. 2003). The Gail Model 2 also forms the basis of the US National Cancer Institute's Breast Cancer Risk Assessment Tool, accessible at http://www.cancer.gov/bcrisktool/.

The Gail Model 2 is most accurate for non-Hispanic White women who receive annual mammograms, but tends to overestimate risk in younger women who do not receive annual mammograms. The model demonstrates reduced accuracy in populations with demographics (age, race, screening habits) that differ from the population on which it was built. At the individual level, the model lacks adequate discrimination in predicting risk and has been challenged on its generalizability across populations (Rockhill et al. 2001). For example, among women participating in the Nurses' Health Study, only 3.3% of 1,354 cases of breast cancer that arose in women within the age-risk strata were defined by the Gail model (Rockhill et al. 2001). Low discrimination, particularly across populations, has spurred numerous efforts to overcome some of the limitations of the previous models (Bondy and Newman 2006). To address concerns regarding applicability of the Gail Model

to African American women, Gail and colleagues have derived a CARE Model using data from a large case control study of African American women participating in the Women's Contraceptive and Reproductive Experiences (CARE) Study (Bondy and Newman 2003; Adams-Campbell et al. 2007). The CARE Model has improved predictive accuracy over the Breast Cancer Risk Assessment Tool, which significantly underestimated risk for African American women. The CARE Model demonstrated high concordance between the numbers of cancer predicted and the number of cancers observed among African American women when validated in the WHI cohort (Gail et al. 2007). Improvements in risk prediction and clinical tools are likely to emerge in the next few years with the addition of such factors as breast density (Barlow et al. 2006), mammographic density change across exams (Kerlikowske et al. 2007a), use of HRT (Santen et al. 2007), and a variety of other factors such as weight, age at birth of first live child, and number of first-degree relatives with breast cancer (Barlow et al. 2006; Chen et al. 2006). Going forward, it is likely that there will be models specifically for risks of premenopausal versus postmenopausal cancers and for specific cancer subtypes (luminal versus basal) (Millikan et al. 2007; Colditz et al. 2004; Chlebowski et al. 2007; Gail et al. 2007; Yang et al. 2007). For example, Chlebowski and colleagues, using data from the WHI cohort, found that the component risk factors of the Gail model and the Gail model itself were more predictive for ER-positive than ER-negative tumors in postmenopausal women where age, family history (first-degree relative) and previous biopsy were sufficient to capture the observed elevation in initial risk (Chlebowski et al. 2007).

## 14.17 Conclusion

The ultimate goal of breast cancer risk assessment and prevention is to individualize clinical management for women at moderate to high risk of breast cancer to extend every woman's life expectancy. At present, scientific evidence supports the integration of prevention counseling and consideration of prevention options (prophylactic surgery or pharmaceutical interventions) for women with strong family histories of breast cancer and for those with personal histories of breast biopsy with proliferative changes (i.e., women at moderate to high risk). Unfortunately, this approach only captures a small number of women who will be affected by a breast cancer diagnosis. Despite the demonstrated efficacy of tamoxifen to prevent invasive breast cancers, unacceptable adverse outcomes (stroke, endometrial cancers and quality of life concerns), combined with a failure to accurately identify women who will most benefit from chemoprevention, limits the clinical use of tamoxifen as a general preventive recommendation. Ongoing and future efforts will hopefully fill these gaps through the identification of better discriminators of risk and identification of benign prevention strategies that will reduce the risk or delay the onset of breast cancer.

Although the mortality and incidence rates due to breast cancer are declining, a major breakthrough for breast cancer will come from the application of the knowledge gained on risk factors and tumor biology to prevention strategies that can be efficaciously and safely administered to a majority of women. Practice guidelines and recommendations for

the individual patient will benefit from the improved accuracy and general application of existing risk models, new prevention agents, and a field ready to rapidly translate advancements to medical practice in a timely and effective manner.

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# Prostate Cancer Prevention

# 15

S. Stratton, F. Ahmann

Research conducted since the 1990s has begun to characterize the molecular pathways involved in carcinogenesis of the prostate. The processes of initiation, cell growth and invasion have begun to be elucidated. In addition, influences of the interactions between cancer cells and their environment that contribute to disease progression are the subject of intense study. Some of these factors include alterations in expression of adhesion molecules that regulate cell–cell and cell–matrix interactions (Mundy 1997; Prasad et al. 1998; Mason et al. 2002; Ross et al. 2002), matrix-metalloproteinase (MMP) expression (Lokeshwar 1999; Zucker et al. 1999) that contributes to the processes of invasion and metastases, hormone independent growth of prostate cancer cells that have become refractory to androgen ablation therapy (Cronauer et al. 2003), and altered expression of proteins that regulate cell proliferation and apoptosis (Johnson and Hamdy 1998; Westin and Bergh 1998).

## 15.1 Prevention of Prostate Carcinogenesis

Prostate cancer, from a prevention perspective, presents several challenges. The rate of occurrence of the steps in carcinogenesis is highly variable. Men begin to develop microscopic foci of prostate cancer in the third decade of life which increase in frequency to such a degree that by a man's eighth decade of life, he has an approximate 67% chance of having microscopic foci of prostate cancer at post mortem exam (Holund 1980). While the vast majority of these microscopic foci do not lead to clinically significant disease, a small percentage of these cancers evolve into aggressive, clinically significant disease that resulted in the diagnoses of 186,320 cases and about 28,660 deaths in 2008 in the United States (US) (Jemal et al. 2008).

Although there has been significant progress that has improved our understanding of the disease, there is still much to be learned about the causes, early diagnostic markers

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prognostic indicators, therapy and prevention. A large part of the challenge for treatment of prostate cancer is our current inability to differentiate between primary tumors that will result in fatal disease from a tumor that will grow very slowly and hence be clinically insignificant. In order to help overcome this challenge, several areas of research must be approached. First, it is critical that we identify genetic, physiological and environmental factors that contribute to increased risk. Second, molecular and cellular processes contributing to development, invasion and the metastasis of prostate cancer must be examined for development of improved early detection methods and targeted therapies. Third, continuous epidemiological studies must be ongoing to understand the relationship of incidence and mortality in different populations and within families. Finally, in vitro and in vivo models of prostate cancer must be developed to facilitate preclinical studies that will lead to development of therapeutic and chemopreventive agents. This chapter will discuss prostate cancer screening, molecular mechanisms that regulate prostate carcinogenesis and agents that show promise for prostate cancer chemoprevention.

In considering strategies to prevent prostate cancer it is important to recognize the disease as a continuum with a long, multistep process in which there are numerous opportunities for intervention (Fig. 1). Therefore, the concept of prevention encompasses primary prevention and prevention of progression of disease. Thus, the true idea of prevention is the treatment or inhibition of the process of carcinogenesis.

Part of the challenge in developing prevention strategies is to identify populations within defined risk groups. In prostate cancer, several risk groups can be defined including: average risk–normal healthy men with normal prostate specific antigen (PSA) levels; high-risk–men who have had either a suspicious digital rectal exam or increased PSA but a normal prostate biopsy; and an even higher-risk group who have had a prostate biopsy negative for cancer, but positive for prostatic intraepithelial neoplasia, a precursor for prostate cancer. Strategies for prevention are being studied in each risk group.

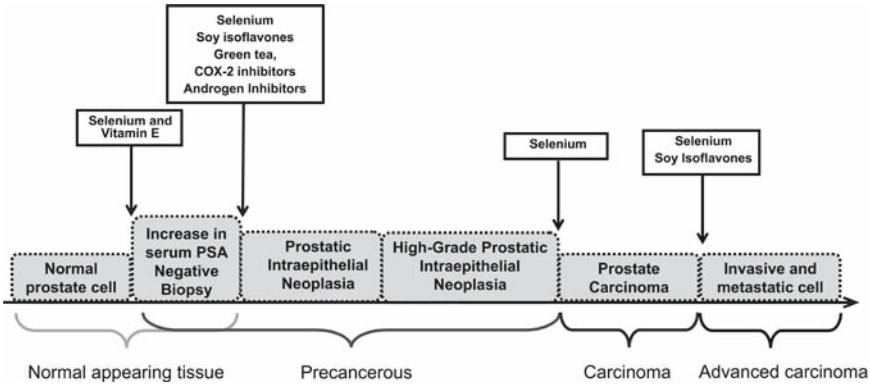


**Fig. 1 The multi-stage process of prostate carcinogenesis.** Cancer prevention encompasses both primary prevention and prevention of disease progression at various stages. Inhibition of disease progression at an early stage of carcinogenesis could prevent development of clinically significant disease thereby decreasing morbidity and mortality associated with prostate cancer. Prostatic intraepithelial neoplasia may provide an ideal target for chemopreventive strategies for prostate cancer

## 15.2 Epidemiology and Risk Factors

Prostate cancer is the second most frequently diagnosed malignancy in men surpassed in incidence only by non-melanoma skin cancers. In 2008, approximately 186,320 men were diagnosed with prostate cancer and an estimated 28,660 men died from the disease in the US (Jemal et al. 2008). Factors associated with increased risk of developing clinically significant prostate cancer include: advancing age, a positive family history, race (African-American), the presence of high-grade prostatic intraepithelial neoplasia, genetics, hormone levels, a digital rectal exam finding an indurated and/or nodular prostate, and an elevated serum level of PSA (Giovannucci and Platz 2002). These risk associations form the basis for chemoprevention strategies in prostate cancer.

The etiology of prostate cancer remains largely a mystery. Epidemiological studies conducted over the past 20 years have revealed statistically significant differences of clinically apparent prostate carcinoma between several economically developed nations (Table 15.1). The most recent estimates of worldwide cancer incidence show that prostate cancer is the third most common cancer in men accounting for nearly 10% of male cancers. Because it is a disease of the elderly, the highest incidences are on developed countries with higher proportions of elderly men. In developing countries, prostate cancer accounts for approximately 4% of cancers in men while in developed countries it accounts for 15% of male cancers (Quinn and Babb 2002).

**Table 15.1.** Prostate cancer incidence in developed countries (cases per 100,000) (Quinn and Babb 2002)

| Country | Cases |
|---|---|
| Australia | 53.5 |
| Canada | 63.0 |
| Denmark | 31.0 |
| Equador | 22.4 |
| England & Wales | 28.0 |
| France | 48.1 |
| Iceland | 61.0 |
| India | 8.0 |
| Israel | 23.9 |
| Japan | 6.8 |
| Netherlands | 39.6 |
| Norway | 48.4 |
| Poland | 15.5 |
| Slovakia | 22.0 |
| Spain | 21.0 |
| Sweden | 55.3 |
| USA | 118.9 |

Within the US, the incidence of prostate cancer is not evenly distributed among men of different racial background and ethnicity or geographical region. Data describing the epidemiology of prostate cancer from the 1930s to the present document a dramatic racial difference in incidence, survival, and mortality rates in American men. African American men have the highest incidence and mortality rates of prostate cancer in the world (Burks and Littleton 1992; Clegg et al. 2002; Carroll 2003). Survival data have been related to access to medical care, genetic and environmental factors, and cultural differences, including diet and social habits. Most reports present conflicting data with no clear positive correlations, and conclusions are often speculative (Burks and Littleton 1992). In addition, while prostate cancer is one of the major malignant diseases in Western countries, in Japan, the incidence and mortality of prostate cancer is remarkably low by comparison; however, it is continuously increasing over time. The increase in incidence within the last 10 years in the Japanese population is speculated to be attributed to the growth of the elderly population, a westernized diet in daily life and widespread environmental contamination of carcinogens. Also, the increase in incidence of prostate cancer in Japan has been attributed to the improvement of screening techniques such as the serum PSA test (Imai et al. 1994). This makes the assumption that the low incidence of prostate cancer was always deceivingly low in this population because it was never detected. Epidemiological studies conducted by Imia and colleagues comparing the incidence of prostate cancer in the United States versus Japan suggested that the strikingly large difference in incidence was also skewed because the prostatic cancers diagnosed in men in the US appeared to be more aggressive than those in men in Japan. The epidemiology of prostate cancer hints that its etiology is both environmental and genetic. Androgenic stimulation over time, perhaps due to a high fat diet, has been suggested as a cause of prostate cancer (Imai et al. 1994, 2004).

## 15.3 Risk Factors

*Age*. The most apparent risk factor of prostatic carcinoma appears to be age. Ninety-five percent of all prostatic cancers occur in men ranging from 45 to 80 years of age. However, before the age of 50, men are at relatively low risk. For men 50–54 years of age, incidence is approximately 30 cases in every 100,000 men. However, beginning at 55 years of age, the potential for developing prostatic carcinoma is heightened dramatically and can even double with each successive 5-year age increment, or increase 1% per year of age resulting in an overall incidence of 1,000 cases per 100,000 men aged 85 years and older (Guinan et al. 1981; Sharifi et al. 1981).

*Race*. The racial disparity observed in prostate cancer incidence and mortality rates among African American and White males in the US is the subject of intense investigation. Various reports, including those of the American Cancer Society (ACS) and the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI), indicate that African American men are at the highest risk for developing prostate cancer with overall mortality rates up to twofold higher than white men (Clegg et al. 2002; Sarma and Schottenfeld 2002) (Table 15.2).

Data adjusted for socioeconomic status and co-morbidities show that African American men are less likely to undergo routine screening for prostate cancer as recommended by the ACS suggesting that greater efforts must be made to advocate screening in this population in order to reduce prostate cancer mortality.

**Table 15.2.** Prostate cancer incidence per 100,000 by race (1996-2002) (NCI 2004)

| Age | Race | Incidence |
|---|---|---|
| All ages | All races | 176 |
| | Caucasian | 168 |
| | African American | 277 |
| < 65 years | All races | 57 |
| | Caucasian | 55 |
| | African American | 102 |
| 65 years | All races | 975 |
| | Caucasian | 947 |
| | African American | 1486 |

Although these statistics indicate a positive race-association in the high incidence of prostate cancer, race is still a debatable indicator of cancer incidence. Numerous reports have examined variations in dietary factors and biological factors, including genetic susceptibility (Schaid 2004) and testosterone levels (Eastham et al. 1998; Winters et al. 2001), however, findings have thus far been inconclusive. Early studies showed promise in identifying candidate regions for prostate cancer susceptibility loci, however replication of the linkage analyses have been challenging (Schaid 2004).

With the intent to reduce the incidence of prostate cancer mortality in African Americans by aggressively increasing the frequency of serum PSA testing, the ACS recommends annual serum PSA measurement for African American men aged 45 years and older as opposed to age 50 for men of other races (ACS 2008). Unfortunately, African American men's adherence to recommended screening intervals is problematic. In addition, access to PSA screening is a crucial determinant of prostate cancer mortality. Studies conducted by Etzioni and colleagues indicated that African American men were 25% less likely to undergo the recommended routine prostate cancer screening by measurement of serum PSA (Etzioni et al. 2002). Clearly, methods to educate the population of interest and advocate serum PSA measurement as recommended by the ACS are a great need and could have a profound effect on prostate cancer mortality in African American men and healthcare economics.

*Prostatic Intraepithelial Neoplasia (PIN)*. Prostate cancers are characteristically heterogeneous and multifocal in nature and can have diverse clinical and morphologic manifestations. Although the molecular basis for this heterogeneity has yet to be elucidated, further study of prostatic intraepithelial neoplasia (PIN), a pathologic diagnosis considered to be a precursor lesion for prostate cancer, may help contribute to characterization of specific cancers. Prostate cancer may arise from other types of precursors, however PIN is considered to be the most likely precursor for prostate cancer at present. The strong genetic similarities between PIN and carcinoma of the prostate suggest that evolution and clonal expansion of PIN leads to development of cancer (Alsikafi et al. 2001; Fowler et al. 2001; Marshall 2001). Data examining age and race as risk factors have revealed that African American men develop more extensive High grade PIN (HGPIN) at a younger age than white men and some studies have shown that patients with PIN will be diagnosed with

prostate cancer within 10 years of their original biopsy (Sakr et al. 1996; Sakr and Partin 2001). It is of interest that pronounced genetic heterogeneity is characteristic of both PIN and carcinoma. Furthermore multiple foci of PIN and carcinoma can arise independently within the same prostate suggesting a field effect of factors that influence carcinogenesis.

HGPIN lesions and prostatic tumors have been shown to share a broad spectrum of molecular and genetic abnormalities including loss within the chromosome regions 8p, 10q, 16q, 18q, and gain within chromosome regions 7q31, 8q (Sakr and Partin 2001). Other abnormalities that have been identified in both HGPIN lesions and prostatic carcinoma tumors include amplification of the oncogene, c-myc (Sakr and Partin 2001), aberrations in nuclear chromatic pattern (Bartels et al. 1998), altered activity of telomerase (Iczkowski et al. 2002), cell cycle regulators (Henshall et al. 2001), proliferative indices, and markers of apoptosis (Johnson et al. 1998; Xie et al. 2000). These data suggest that HGPIN is an intermediate stage between benign prostatic epithelium and prostatic carcinoma and it may be critical in early stages of carcinogenesis and neoplastic progression in the prostate. HGPIN could therefore potentially serve as an ideal target for early diagnosis of prostate cancer and development of agents to prevent progression of early stages of carcinogenesis.

In studying HGPIN as a precursor to prostate cancer, it is important to take into account that endocrine therapy causes alterations in morphology of PIN making it more closely resemble normal prostatic epithelium (Bostwick 2000). Endocrine therapy-induced changes in molecular markers of PIN and induction of resistance to endocrine therapy are currently important areas of study.

*Genetic Factors*. More recently, genetic factors that contribute to prostate cancer have been examined. Research into the molecular genetics of prostate cancer to date has largely focused on the possible existence of one or several single-locus high-penetrance susceptibility genes and several candidate regions have been identified, but confirmatory studies of these regions have been inconclusive. Increasingly, attention has turned to identification of candidate genes that may increase prostate cancer risk because their products potentially play an important role in possible etiological pathways for prostate cancer (Ostrander and Stanford 2000; Xu et al. 2000). Of various such pathways that have been suggested for prostate cancer, the best studied in terms of molecular genetics is the androgen signaling pathway. Two genes in this pathway, the androgen receptor (AR) gene and the steroid 5-alpha reductase type II (SRD5A2) gene, have been under particular scrutiny and polymorphic markers in each of these genes which reproducibly predict prostate cancer risk have been identified (Abate-Shen and Shen 2000).

Abnormalities in chromosomes 8 and 10 may be associated with development of prostate cancer. Preliminary findings by Kunimi and colleagues in 1991, using restriction fragment length polymorphism analyses (RFLP), showed consistent alterations in genetic information located in chromosomes 8 and 10 in men with prostate cancer (Kunimi et al. 1991). Subsequent studies of alterations in chromosomes 8 and 10 have been completed. NCI-sponsored studies demonstrated that a deletion in chromosome 8 (8p21) was present in 80% of prostate cancers and approximately 63% of precancerous prostate lesions (Kagan et al. 1995; Qian et al. 1997; Katoh 2002b). Such findings suggest that abnormalities in chromosome 8p21 may be associated with early development of prostate cancer and changes in protein expression associated with this deletion may serve as early diagnostic markers or targets for treatment of early prostate carcinogenesis. Studies conducted by Katoh and colleagues showed downregulation in primary prostate cancer and prostate car-

cinoma cell cultures, of the SOX7 gene, located on chromosome 8p22 (Katoh 2002a,b). This gene is thought to be a tumor suppressor gene for prostate cancer and other solid tumors including kidney and breast. Other genes identified that are potentially involved with the carcinogenesis of prostate cancer include LZTS1 located on chromosome 8p22 (Cabeza-Arvelaiz et al. 2001) and KLF6 (Narla et al. 2001), located on chromosome 10p.

## 15.4 Screening

The methods used to detect prostate cancer underwent a dramatic change with the introduction of serum PSA levels as a screening test in the late 1980s. Prior to the late 1980s, men were selected for a prostate biopsy almost entirely on the basis of them having a suspicious feeling prostate on a digital rectal exam (DRE). Beginning in the late 1980s, studies demonstrated the serum levels of PSA had a higher sensitivity and specificity for identifying men who would be found to have prostate cancer on needle biopsies than did the results of DRE (Catalona et al. 1994). The use of serum PSA levels as a prostate cancer screening test dramatically increased the number of prostate needle biopsies performed in the US, and dramatically increased the number of men diagnosed with prostate cancer. Recent mortality figures demonstrate a sharp and continuing decline in prostate cancer mortality since the middle 1990s, approximately 6 years after screening with serum PSA levels started to be used in clinical practice (Bishop 2000; Quinn and Babb 2002; Stephenson 2002). The most rational explanation for this decline in prostate cancer mortality is the successful use of PSA screening leading to the earlier diagnosis of prostate cancer and the application of potentially curative therapies (radical prostatectomies or radiotherapy).

A second change occurred in how prostate cancers were being diagnosed during this same time period. In the 1980s, the standard prostate needle biopsy technique was accomplished using a digitally guided needle via a transrectal approach. It was recognized in the late 1980s that this biopsy technique was missing prostate cancers that occurred in areas of the gland that were palpably normal (Catalona et al. 1994). Needle biopsies of the prostate performed under transrectal ultrasound guidance were shown to increase the number of cancers found (Presti 2002). Using this method, the prostate biopsies could now sample the prostate in a reproducible and uniform manner. The technique evolved from a four-quadrant biopsy (two biopsy cores on each side of the prostate) to a standard sextant biopsy (three biopsy cores on each side of the prostate) by the early 1990s. More recently, additional biopsy cores taken from the lateral horns of the prostate have been shown to improve the detection of prostate cancers (Taylor et al. 2002).

A dilemma that previously had attracted little attention became recognized as prostate biopsy techniques and screening techniques evolved. Some men had either persistent elevations of serum PSA levels in the face of a negative prostate biopsy or had persistent palpably abnormal prostates despite a negative prostate biopsy. Multiple reports have now been published after performing second biopsies in such men. Table 15.3 shows 20 studies published since 1993 utilizing sextant (or more) second (or more) biopsies of the prostate in men previously biopsied and having no evidence of prostate cancer. The studies displayed are confined to those conducted in the US and Western Europe to minimize any effect from the well-

**Table 15.3.** Frequency of prostate biopsy following an initial negative prostate biopsy

| Reference | Number of 2nd (or more) biopsies | Number (% of total) positive |
|---|---|---|
| (Andriole and Catalona 1993) | 73 | 30 (41%) |
| (Keetch, Catalona et al. 1994) | 427 | 104 (24%) |
| (Ellis and Brawer 1995) | 100 | 20 (20%) |
| (Hzyek 1995) | 51 | 8 (16%) |
| (Lui, Terris et al. 1995) | 187 | 72 (38%) |
| (Roehrborn, Gregory et al. 1996) | 123 | 30 (24%) |
| (Rovner, Schanne et al. 1997) | 71 | 17 (24%) |
| (Perachino, di Ciolo et al. 1997) | 148 | 60 (41%) |
| (Fleshner, O'Sullivan et al. 1997) | 130 | 39 (30%) |
| (Ukimura, Durrani et al. 1997) | 193 | 51 (26%) |
| (Rietbergen and Schroder 1998) | 442 | 49 (11%) |
| (Letran, Blase et al. 1998) | 51 | 15 (29%) |
| (Levine, Ittman et al. 1998) | 137 | 43 (31%) |
| (Durkan and Greene 1999) | 48 | 15 (31%) |
| (Djavan, Zlotta et al. 2000) | 820 | 83 (10%) |
| (Borboroglu, Comer et al. 2000) | 57 | 17 (30%) |
| (Kamoi, Troncoso et al. 2000) | 45 | 10 (22%) |
| (Fowler, Bigler et al. 2000) | 298 | 80 (27%) |
| (Stewart, Leibovich et al. 2001) | 224 | 77 (34%) |
| (Park, Miyake et al. 2003) | 104 | 22 (21%) |
| **Summary** | **3729** | **842 (23%)** |

recognized global variability in the incidence of prostate cancer. The positive second biopsy rate amongst these studies ranges from 10 to 41%. Overall, a total of 3,729 second biopsies were carried out and 842 (23%) were positive. No consistent criteria were utilized in these studies to determine when a second biopsy was done. However, in the vast majority of the series, the reason for a second biopsy was the persistence of an elevated serum PSA level. This correlates well with the usual clinical practice in the United States. Various authors have proposed tools to increase the specificity of a second biopsy including free versus bound PSA ratios, PSA density, age adjusted PSAs, and PSA velocities, but none are definitive.

## 15.5 Molecular Markers of Prostate Carcinogenesis

Elucidation of the molecular mechanisms involved with each stage of carcinogenesis is one of the first steps in identifying targets for chemoprevention strategies; however, the apparent multiplicity of molecular factors in prostate carcinogenesis has proven to be a

challenge to decipher. While some genetic abnormalities that result in aberrant gene expression are hereditary, all tumors acquire additional abnormalities in gene expression as the carcinogenesis progresses. Overexpression of genes can be caused by several mechanisms including gene amplification, a mutation within the promoter region or upregulation of an upstream signaling factor. Similarly, decreased expression of tumor suppressor genes can be caused by a promoter region mutation, deletion or by gene silencing due to promoter methylation. Several cell signaling factors have been identified as potential markers for early diagnosis and as possible targets for chemoprevention. These factors include oncogenes, tumor suppressors and proteins involved with inflammation.

*Androgens*. Testosterone and 5α-dihydrotestosterone are the two most abundant androgens (Fig. 2). Both act through one androgen receptor although each androgen plays specific roles during male sexual differentiation. Testosterone is directly involved in the development of structures derived from the wolffian duct (epididymides, vasa deferentia, seminal vesicles and ejaculatory ducts), while 5α-dihydrotestosterone, a metabolite of testosterone, is the active ligand in most androgen-sensitive target tissues including the urogenital sinus and tubercle and their associated structures such as the prostate and urethra. Each isoform interacts with the androgen receptor differently. Testosterone lower affinity for the receptor and a higher dissociation rate compared to 5α-dihydrotestosterone (Schiavi and White 1976; Knol and Egberink-Alink 1989; Vermeulen 1991). This requires that testosterone be present in very high concentration within the tissues in which they act to elicit its downstream signaling.

The biosynthetic conversion of cholesterol to testosterone occurs through several intermediates. The first step entails transfer of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR) and the subsequent P450scc-mediated side chain cleavage of cholesterol (Aspden et al. 1998). This conversion, resulting in the synthesis of pregnenolone, is a rate-limiting step in testosterone biosynthesis. Subsequent steps require several enzymes including 3b-hydroxysteroid dehydrogenase, 17a-hydroxylase/C17–20-lyase and 17b-hydroxysteroid dehydrogenase (Dessypris 1975; Panaiotov 1978).

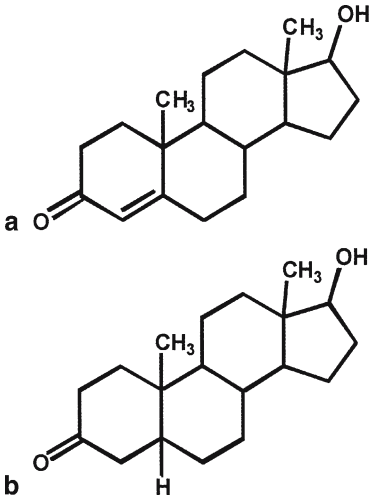


**Fig. 2** Structure of (**a**) testosterone and (**b**) 5α-dihydrotestosterone

*Androgen Receptor Polymorphisms*. The androgen receptor (AR) is a member of the steroid/nuclear receptor gene superfamily. The encoding gene is located on chromosome loci Xq11.2–q12 and consists of eight exons. There are two domains that are directly responsible for the transactivation activity of the AR protein. Of these domains, the ligand-independent AF-1 is encoded within exon 1.

There are three known AR gene polymorphisms which may account for variations in risk for prostate cancer. These polymorphisms include the (CAG)n trinucleotide repeat, the (GGC)n trinucleotide repeat, and the R726L single nucleotide polymorphism (Mononen et al. 2000). Studies conducted by Irvine et al. suggested that there are 27 observed alleles in various populations with repeats ranging from 5 to 31. Short CAG repeats (less than or equal to 22 repeats) were found to be more prevalent in African-American males who are at high risk for prostate cancer and less prevalent in Asians who are at lower risk for this disease (Irvine et al. 1995). These findings suggest that variations in androgen receptor CAG repeat length differs considerably among human populations and may account for variations in risk. African-Americans also had the lowest frequency (20%) of the GGC allele with 16 repeats compared to intermediate-risk whites and low-risk Asians which showed frequency of this sequence 57% and 70%, respectively. In an Australian case control study performed by Beilin and colleagues on 545 cases of prostate carcinoma and 456 age-matched controls, the odds ratio of prostate carcinoma for a change of five CAG repeats was 0.98 (95% confidence interval [CI]: 0.84–1.15); therefore suggesting that the *AR* CAG repeat polymorphism was not a risk factor for prostate carcinoma in this population. However, in this study, a shorter repeat sequence was found to be associated with earlier age at diagnosis (Beilin et al. 2001).

In another study conducted in China, Hsing and colleagues showed that Chinese men have longer CAG (equal or longer than 23) repeats compared with US men. It is of interest that this study also suggests that even in a relatively low-risk population, a shorter CAG repeat length is associated with a higher risk of clinically significant prostate cancer. Chinese men with a CAG repeat length shorter than 23 (median length) had a 65% increased risk of prostate cancer (odds ratio [OR]=1.65; 95% CI: 1.14–2.39) (Hsing et al. 2000). In contrast to the previously described studies examining polymorphisms in sporadic prostate cancer, research conducted by study by Miller and colleagues suggest that the (CAG)n and (GGN)n repeats do not play a major role in familial prostate cancer (Miller et al. 2001). Clearly, larger studies are needed to evaluate the combined effect of CAG and GGN repeats. Because both genetic and environmental factors contribute to cancer risk, future studies should incorporate biomarkers of environmental exposures as well as AR gene polymorphisms.

*Nuclear Factor Kappa B (NFκB)*. NFκB is an ideal example of a potential target and biomarker for prostate cancer chemoprevention. The NFκB/Rel family of proteins in mammals consists of five members: c-Rel, NFκB1 (p50/p105), NFκB2 (p52/p100), Rel A (p65), and Rel B, all of which share a matched Rel homology domain (RHD). The NFκB proteins function as a variety of heterodimers, allowing structural and functional versatility (Baldwin 1996). The active form of NFκB, commonly comprised of p50 and p65 subunits of the Rel family of proteins, is known to regulate genes involved in apoptosis, cell cycle arrest and inflammation (Witkamp and Monshouwer 2000; Sun and Andersson 2002). The dimeric protein is sequestered in the nucleus by its inhibitory subunit, IκB. In order to become activated, a cascade of events involving recruitment enzymes and kinases

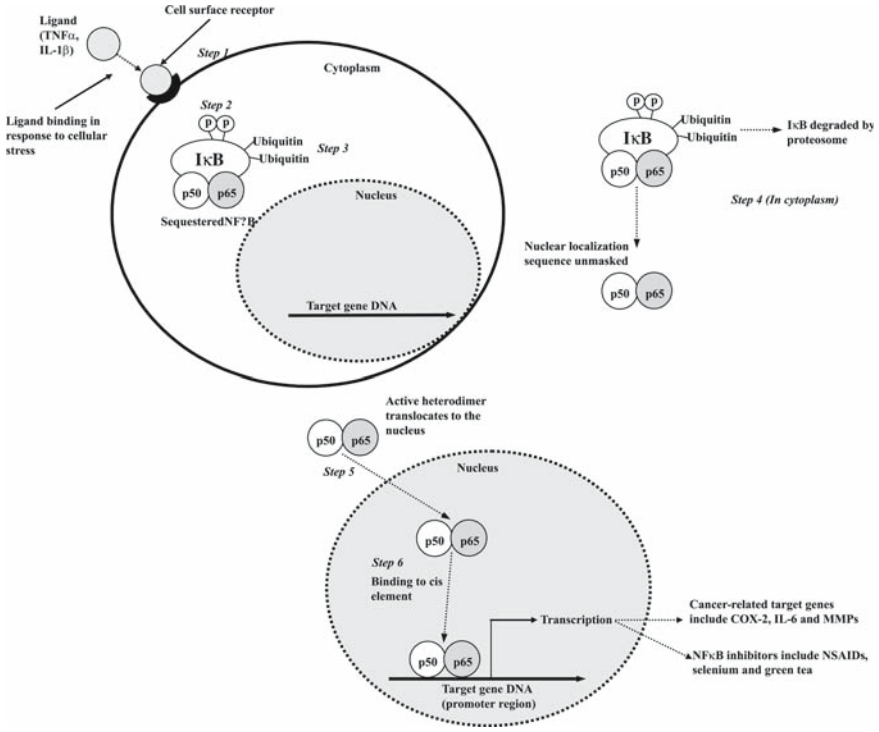


**Fig. 3 Regulation of NFκB transactivation activity.** NFκB is sequestered in the nucleus by IκB. Upon ligand binding to an extracellular receptor, a cascade of events results in phosphorylation and ubiquitination of IκB followed by degradation by the 26S proteosome. Once the nuclear localization sequence on NFκB is unmasked, it can translocate to the nucleus and bind the promoter region of target genes

must occur (Fig. 3). In response to an NFκB regulatory ligand such as IL-1b or TNFα binding to its respective cell surface receptor, the IκB subunit is phosphorylated at two serine residues and is subsequently ubiquitinated and degraded by the 26S proteosome. This unmasks the nuclear localization sequence located on the p50 subunit of the heterodimer thus allowing the activated NFκB complex to be translocated to the nucleus where it can then bind *cis* elements within the promoter regions and elicit transactivation of target genes (Sun and Oberley 1996; Mercurio and Manning 1999). This complex mechanism of activation provides several points at which NFκB transactivation activity can be inhibited.

NFκB is frequently overexpressed and constitutively activated in carcinomas and is a key antiapoptotic factor in most mammalian cells (Sellers and Fisher 1999; Bours et al. 2000). Furthermore, NFκB is overexpressed in prostate cancer tissue models derived from metastatic tumors which provides further evidence of its role in cancer progression. Increased activity of NFκB is of particular interest in prostate cancer because some of its downstream genes, including IL-6 and cyclooxygenase-2 (COX-2), play a role in prostate carcinogenesis. Upstream events in the NFκB activation cascade have become of interest as chemotherapeutic and chemopreventive molecular targets. Some agents, including

inhibitors of COX enzymes (Surh et al. 2001), have been shown to block phosphorylation of IκB causing inhibition of NFκB activation, while other agents, such as the p50 binding peptide produced by Calbiochem (San Diego, CA), block the nuclear localization sequence of the active NFκB heterodimer (Maliner-Stratton et al. 2001). This mechanism allows dissociation of NFκB from the inhibitory subunit, but blocks nuclear translocation and, therefore, NFκB transactivation activity.

*NFκB and Selenium*. Several studies have shown that one of the mechanisms by which selenium may elicit anticancer activity may be by inhibition of NFκB transactivation activity. Jiang and colleagues demonstrated that selenium compounds inhibited cell growth and induced apoptosis in human prostate carcinoma cell lines through an NFκB-dependent mechanism (Jiang et al. 2001; Gasparian et al. 2002). The effects of methylselenic acid (MSeA), a novel precursor of methylselenol, were compared with sodium selenite on apoptosis, cell cycle arrest and MAP kinase activity on the hormone refractory prostate cancer cell line, DU-145. The agents tested inhibited TNFα-induced NFκB transactivation activity and blocked transcription of several components of the cascade responsible for activation of the NFκB heterodimer. Furthermore, exposure of DU-145 cells to 3 lM MSeA induced cell cycle arrest in G1 after 24 h; and exposure to higher concentrations resulted in DNA fragmentation and caspase-mediated cleavage of poly(ADP-ribose) polymerase (PARP), two standard hallmarks of apoptosis.

Stable transfection of a prostate carcinoma cell line with a dominant negative IκB caused sensitization to selenium (Se)-induced apoptosis further suggesting that NFκB may be a primary mechanism for the chemopreventive effects of Se compounds (Gasparian et al. 2002). Sodium selenite and MSeA, an organic form of Se, have also been shown to block NFκB activation in the prostate carcinoma cell line, JCA1. Both compounds inhibited NFκB transactivation activity in cells transfected with a plasmid construct encoding a luciferase reporter gene driven by a minimal promoter with NFκB *cis* elements (Gasparian et al. 2002). Clearly, NFκB may serve as an ideal target not only for prostate cancer chemoprevention, but also for treatment of advanced disease.

*Interleukin-6*. IL-6, which was initially named B cell differentiating factor or hepatocyte stimulating factor, is produced by many cell types including T cells and B cells (Oleksowicz and Dutcher 1994; O'Shaughnessy et al. 1996), macrophages (Bost and Mason 1995), fibroblasts (Carty et al. 1991; Raap et al. 2000) and endothelial cells (Jirik et al. 1989; Soderquist et al. 1998). IL-6 is a pleiotropic cytokine downstream of NFκB. The majority of human cell types are IL-6 responsive, however, it plays a particularly important role in differentiation of B cells to mature antigen presenting cells. In hepatic cells, IL-6 regulates expression of acute immune response phase proteins (Hilbert et al. 1995).

Immunohistochemical analyses of frozen tissue sections collected from radical prostatectomies have shown that IL-6 expression is expressed both in the epithelium and stroma, whereas in normal tissue expression is confined to the basal cells of the epithelium (Royuela et al. 2004). IL-6 is thought to be involved in progression of prostate cancer at later stages of carcinogenesis and it may therefore be a valuable surrogate marker for androgen-independent prostate cancer. It is of particular interest that several studies have shown that circulating levels of IL-6 are elevated in patients with hormone refractory prostate cancer and that these levels correlate with tumor burden as assessed by PSA or clinically identified metastases (Wise et al. 2000). In addition, IL-6 signaling is required for bone differentiation which

further indicates this cytokine as a significant contributor to prostate carcinogenesis due to the propensity of prostate cancer to metastasize to bones of the pelvis and spine. Moreover, NFκB-mediated IL-6 signaling is upstream of expression MMPs, which are involved with degradation of the extracellular matrix during tumor invasion.

In vitro studies have begun to elucidate the role of IL-6 in prostate carcinogenesis. Cell lines established from androgen-responsive tumors do not endogenously express IL-6 however these cells will become androgen independent when treated with IL-6 (Lee et al. 2003c). Prostate cancer cell lines unresponsive to androgens that are established from bone and brain metastases express IL-6 and are also androgen independent (Lee et al. 2003a). Although hormone-responsive prostate cancer cell lines do not constitutively express IL-6, they can be induced to express IL-6 through an NFκB-dependent mechanism. Studies conducted in our laboratory showed that LNCaP cells secrete IL-6 in response to IL-1β resulting in expression of the MMP, matrilysin, which is involved with degradation of the extracellular matrix during invasion and metastases (Maliner-Stratton et al. 2001). These data suggest that IL-6 may play a role in the point of prostate carcinogenesis at which the cancer cells become androgen independent making it a viable candidate for prevention progression of prostate cancer to an invasive phenotype.

*IL-6 Signaling in Prostate Cancer Cells*. IL-6 frequently elicits its downstream effects by signaling through the transcription factor, signal transducer and activator of transcription 3 (STAT3). Binding of IL-6 to its receptor leads to activation of Janus kinases as well as two major downstream signaling components, STAT3 and MAPK in the prostate carcinoma cell line, LNCaP (Spiotto and Chung 2000a,b). STAT3 has been shown to mediate neuroendocrine differentiation of LNCaP cells. In addition, in the differentiated cells showing neurite outgrowth and increased expression of the neuroendocrine markers, neuron specific enolase and chromagranin A that had undergone growth arrest, STAT3 remained active (Spiotto and Chung 2000). Chung and colleagues also demonstrated that STAT3 mediated IL-6-induced growth inhibition in LNCaP cells (Spiotto and Chung 2000a). It is of interest that the more progressed and less differentiated prostatic carcinoma cell lines, PC3 and DU-145, express a constitutive level of secreted IL-6, however, those data also showed that the less progressed and hormone responsive LNCaP cells do not secrete any detectable IL-6 (Chung et al. 1999). In addition, it has been shown that the more progressed prostatic carcinoma cell lines lack a functional STAT3 pathway (Mori et al. 1999). Research conducted by Ni and colleagues demonstrated that cells derived from both rat and human prostate cancers have constitutively activated STAT3; and STAT3 activation was directly correlated with malignant potential (Ni et al. 2000). Inhibition of STAT3 transactivation activity by ectopic expression of a dominant-negative STAT3 in human the prostate cancer cells significantly suppresses their growth in vitro and their tumorigenicity in vivo. Furthermore, the Janus kinase inhibitor, tyrphostin AG490, inhibited the constitutive activation of STAT3 and suppressed the growth of human prostate cancer cells in vitro. These results indicate that activation of STAT3 signaling is essential in the progression of prostate cancer cells and suggest that targeting STAT3 signaling may yield a potential early therapeutic intervention for prostate cancer.

*Cyclooxygenase-2 (COX-2)*. The COX enzymes catalyze the rate-limiting steps in the conversion of arachidonic acid to proinflammatory prostaglandins. The COX enzyme exists in two isoforms, COX-1 and COX-2. The COX-1 isoform is constitutively expressed in a majority of human tissues and regulates production of prostaglandins that mediate renal

and platelet function, and maintenance of the gastrointestinal mucosa (Oshima et al. 1993). Conversely, COX-2 isoform is not expressed in normal tissues, but is rapidly induced by inflammatory cytokines, growth factors, oncogenes and tumor promoters (Oshima et al. 1996; Reddy and Rao 2000). The two isoforms share 60% overall amino acid identity and 75% core sequence identity; and the sizes of the enzymes are comparable (71 kD). However, despite strong similarities in kinetic parameters, binding sites for arachidonic acid, active sites for NSAID binding, and molecular structure (Thun et al. 1993), the two isoforms differ in substrate affinities due to a single amino acid substitution (isoleucine in COX-1 for valine in COX-2) (Moody et al. 1998) in the NSAID binding site resulting in a void volume to the other side of the central active site channel in COX-2. Compounds designed to bind in this additional space are potent and selective inhibitors of COX-2. The advantage of selective COX-2 inhibitors such as celecoxib and rofecoxib is that they are effective in inhibiting inflammation without blocking the COX-1 enzymes that are important for the maintenance of gastrointestinal tract homeostasis and platelet function.

Overexpression of COX-2 in prostate cancer cells is associated with resistance to apoptosis (Tang et al. 2002). Furthermore, selective inhibition of COX-2 can increase apoptotic index in prostate carcinoma cells in vitro (Fosslien 2001; Kirschenbaum et al. 2001). These observations concur with data that suggest that COX-2 inhibitors could be effective chemopreventative agents. Lim and colleagues showed that the COX inhibitor sulindac, a NSAID frequently used for chronic inflammatory diseases, was tested for pro-apoptotic activity in the prostate cancer cell lines, PC3 and LNCaP, and a normal prostate epithelial cells line, PrEC, in vitro. Apoptosis was quantified following treatment with either sulindac or an active sulindac metabolite, Exisilund (sulindac sulfide). After 48 h, 50% of PC3 cells and 40% of LNCaP cells underwent apoptosis while PrEC cells showed no indication of apoptosis at similar concentrations of drug (Lim et al. 1999).

Studies evaluating the effects of COX-2 specific inhibitors on angiogenesis in prostate carcinoma cell lines have also been performed. The prostate carcinoma cell lines, LNCaP and PC3 and a normal prostate stroma cell line, PrEC were treated with two COX-2 specific inhibitors, Edolac and NS398. Both compounds decreased cell proliferation in the carcinoma cell lines, but not in the normal prostate stromal cell line. A DNA fragmentation assay revealed that both compounds also induced apoptosis in the two carcinoma cell lines and not the normal stromal cell line (Liu et al. 1998).

*B-Cell Lymphoma/Leukemia 2 (Bcl-2)*. Bcl-2 is a member of the bcl-2 family of apoptotic regulatory gene products of the proto-oncogene, b-cell lymphoma/leukemia. bcl-2 family members have been characterized as exhibiting both pro-apoptotic and antiapoptotic properties (Tsujimoto and Croce 1986). Numerous studies have suggested that bcl-2 mediated decrease in apoptotic index plays a role in prostate carcinogenesis (Reed et al. 1996; Gross et al. 1999); and increased expression of bcl-2 and related apoptotic pathways appear to affect the sensitivity of prostate cancer cells to several therapeutic modalities including androgen ablation and radiation. The increase in cell survival following treatment is thought to contribute to development of the androgen-independent phenotype (Apakama et al. 1996; Rosser et al. 2003). Immunohistochemical analyses of prostate tissue have shown overexpression of bcl-2 in androgen-independent carcinoma tissue, but not in androgen-responsive cancers tissue and HGPIN suggesting that bcl-2 is involved in disease progression and not in the early stages of carcinogenesis (McDonnell et al. 1992;

Raffo et al. 1995; Apakama et al. 1996). Retrospective studies of prostate cancer patient survival following radiation therapy have shown that overexpression of bcl-2 was correlative to poor prognosis (Pollack et al. 2003). Additional investigation of bcl-2 in cancerous growth in the peripheral and transitional zones of the prostate have shown a higher incidence of bcl-2 in the highly proliferative peripheral zone when compared to the less proliferative transitional zone in the prostate (Erbersdobler et al. 2002).

The emergence of novel and sensitive techniques such as tissue microarray (TMA) has presented both supportive and negating evidence for bcl-2 as an effective biomarker of prostate cancer progression (Merseburger et al. 2003; Zellweger et al. 2003). Elevated levels of bcl-2 have been observed in prostate cancer tissue following both androgen ablation and radiotherapy. This correlates with in vitro studies demonstrating that bcl-2 elicits a protective effect on cancer cells in response to radiation. The role of bcl-2 in the prevention of apoptosis is critical in the development of prostate cancer recurrence and androgen-independent survival. In this sense, bcl-2 is an encouraging prognostic molecular biomarker.

## 15.6 Prevention Strategies

### 15.6.1 Androgen Inhibitors

In the early 1990s, a prostate cancer prevention clinical trial was designed to test whether the 5 alpha reductase inhibitor finasteride could reduce the number of prostate cancers found in men over the age of 55. The rationale for this strategy was straight forward. Prostate cancer is the leading cause of cancer-related deaths in men over the age of 50 and at the time the trial was designed over 40,000 men were dying each year of prostate cancer. Hormonal factors were targeted for prevention because the only major risk factors identified for prostate cancer development are being male and having normal testosterone production. In the trial planning, various hormonal interventions were considered even including surgical or medical castration but the morbidity of such interventions precluded their use in a prevention study. The debate finally centered on either the use of an antiandrogen which acts via binding to the androgen receptor or the use of finasteride which blocks the conversion of testosterone to another androgen, dihydrotestosterone, which is the most physiologic active androgen acting on the prostate. Finasteride was selected for trial based on its lower toxicity potential. The main potential toxicities known for finasteride were a reduction semen volume, erectile dysfunction, reduced libido and gynecomastia.

The study design was a placebo-controlled randomization of approximately 25,000 men to receive either finasteride 5 mg each day orally or a placebo finasteride pill. Treatment was for 7 years with yearly digital rectal exams and serum PSA levels and quarterly contacts for documentation of medical events and/or toxicities. The identification of a suspicious digital rectal examination or an elevated serum PSA level resulted in a recommendation to have a prostate biopsy. At the end of the 7 years, all men were to be biopsied. It was anticipated that the placebo group would be found to have a 6% incidence of prostate cancer.

Enrollment was completed by May 1997 and the results of the trial were published in 2003 (Thompson et al., 2003). The results were perplexing. The first result was expected and was that the finasteride was reasonably well tolerated with less than 15% of men experiencing the anticipated sexual side effects. The second finding was a significant reduction in the number of cancers found on the end of biopsy study for the men who had been randomized to finasteride. Of those receiving placebo, 24.4% were found to have prostate cancer on the biopsy versus only 18.4% of the men taking the finasteride ($p<0.001$). This 24.8% reduction in the prevalence of prostate cancer marked only the second prevention trial where a pharmacologic intervention reduced the incidence of a cancer (the other being tamoxifen in breast cancer prevention).

There were two other findings of note. First, the histologic grade (the Gleason score) of the cancers found in the men who were taking finasteride were significantly more likely to be a higher grade (Gleason score of 7, 8, 9 or 10) and hence of poorer prognosis than the men who took the finasteride placebo (37% versus 22.2%, $p<0.001$). Since the end point of the study was the incidence of cancer found and not survival, how these conflicting findings (a lower incidence versus a higher histologic grade) effected outcome is not known. The other unexplained finding was an overall incidence of cancer in the placebo group that was four times the anticipated incidence of prostate cancer that the study design called for. The significance of this unanticipated finding is also not known.

Overall, the study demonstrated the potential of chemoprevention in prostate cancer. However, the conflicting and unexplained findings are such that the routine use of finasteride as a chemopreventive agent in prostate cancer is not recommended.

### 15.6.2 Selenium and Vitamin E

Epidemiologic studies have suggested an inverse relationship between intake of dietary selenium and incidence of cancer (Clark and Jacobs 1998; Overvad 1998; Nelson et al. 2002). Numerous animal studies have demonstrated that dietary supplementation with selenium reduces cancer incidence in animal models including melanoma and cancers of the colon, breast, liver, esophagus, head and neck, pancreas, kidney and lung. One of the central findings of the Nutritional Prevention of Cancer study conducted at the University of Arizona, was a greater than 60% reduction in the incidence of prostate cancer in participants randomized to 200 mcg per day of selenium compared to a placebo-treated group (Clark et al. 1996; Duffield-Lillico et al. 2003). An analysis of an additional 3 years of the blinded phase of this study showed a continued significant decrease in prostate cancer incidence in participants within the lowest two tertiles of baseline plasma selenium levels. In these groups, the prostate cancer incidence after 13 years of follow-up was decreased by 86 and 67%, respectively, in the treatment group versus placebo (Duffield-Lillico et al. 2003). These findings led to the development of additional, randomized, blinded, placebo-controlled clinical studies testing the effects of selenium on prevention of primary and secondary prostate cancer. Selenium is an ideal example of a potential chemopreventive agent being tested for efficacy in all risk groups and in every stage of prostate carcinogenesis (Fig. 4).

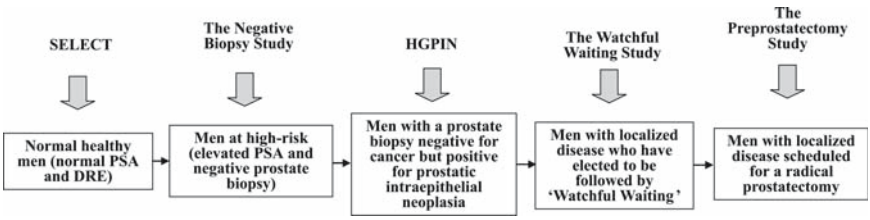
SELECT
The Negative Biopsy Study
HGPIN
The Watchful Waiting Study
The Preprostatectomy Study
Normal healthy men (normal PSA and DRE)
Men at high-risk (elevated PSA and negative prostate biopsy)
Men with a prostate biopsy negative for cancer but positive for prostatic intraepithelial neoplasia
Men with localized disease who have elected to be followed by 'Watchful Waiting'
Men with localized disease scheduled for a radical prostatectomy

**Fig. 4 Selenium and prostate cancer prevention clinical trials.** Selenium provides an ideal example of an agent being tested for chemopreventive activity in all stages of prostate carcinogenesis

*Selenium and Prostate Cancer Prevention in Normal Healthy Men (SELECT Trial)*. The Selenium and Vitamin E Cancer Prevention Trial (SELECT) is an NCI-sponsored randomized, prospective, double-blind study designed to determine whether selenium and/or vitamin E decrease the risk of prostate cancer in healthy men. SELECT is being coordinated by the Southwest Oncology Group (SWOG) and plans to enroll a total of 32,400 normal, healthy men at over 400 clinical study sites in the United States, Puerto Rico and Canada (Klein et al. 2000, 2001). Preclinical, epidemiological and phase III data imply that selenium and vitamin E have potential efficacy for prostate cancer prevention. The four arms of this study include: (1) selenium plus vitamin E; (2) selenium plus placebo; (3) placebo plus vitamin E; and (4) double placebo. Enrollment began in July 2001 and the trial is expected to be completed in 2013. The dose levels of selenium and vitamin E are 200 mcg and 400 mg, respectively (Fig. 5a). In addition to primary and prespecified secondary endpoints, including development of biopsy-proven prostate cancer and PSA velocity, additional tertiary/ancillary endpoints, including dietary/nutrient assessments, pathology and molecular/cellular biomarkers, quality of life, and molecular epidemiology will be evaluated.

*Selenium and Prostate Cancer Prevention in High-Risk Men (The Negative Biopsy Study)*. In addition to recruiting participants to SELECT, the Arizona Cancer Center at the University of Arizona is conducting four clinical studies examining the effects of selenium on primary and secondary prevention of prostate cancer in several populations (Stratton et al. 2003a). The Negative Biopsy Study is an NCI-sponsored study in which 700 men, who have had at least one negative prostate biopsy within 1 year of study enrollment, were randomized to receive either 200 or 400 mcg per day of selenium versus placebo (Fig. 5b). Endpoints include PSA velocity, development of biopsy-proven prostate cancer and serum markers including alkaline phosphatase and chromagranin A. Changes in prostate tissue biomarkers are also being examined.

*Selenium and Prostate Cancer Prevention in Men with Prostatic Intraepithelial Neoplasia (The HGPIN Study)*. While the recognized risk factors for prostate cancer (male gender, a positive family history, age, and the presence of androgens) pose targets for prevention efforts, another potential target includes premalignant lesions. While somewhat controversial, high-grade prostatic intraepithelial neoplasia (HGPIN) is generally accepted to be a premalignant lesion for prostate cancer when found on a prostate biopsy that does not otherwise have evidence of cancer. At the very least, the presence of HGPIN is

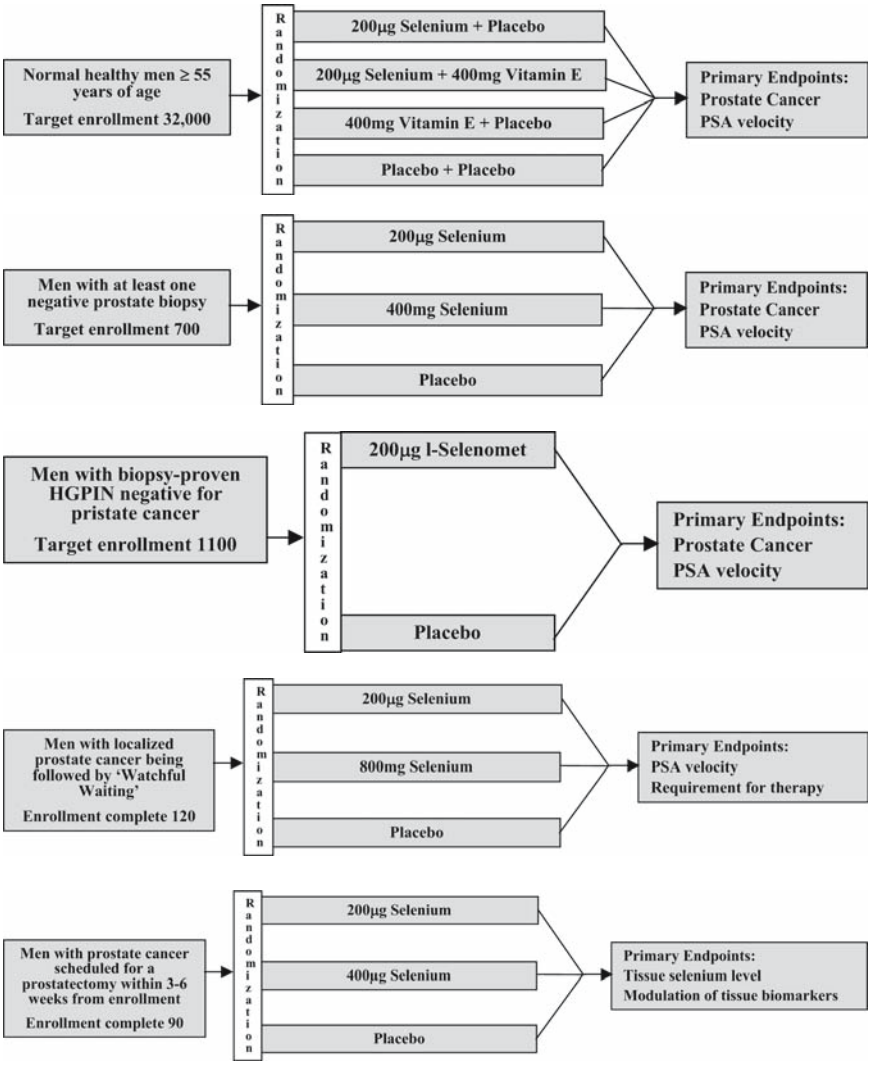


**Fig. 5 Clinical trials:** (a) SELECT is testing the effects of selenium on prostate cancer prevention in normal, healthy men aged 55 years and older; (b) participants enrolled in the Negative Biopsy Study have had at least one prostate biopsy negative for HGPIN and cancer; (c) the HGPIN study is enrolling men with a prostate biopsy negative for cancer but positive for HGPIN; (d) the Watchful Waiting Study is enrolling men with biopsy-proven prostate cancer who have elected to forgo therapy and be followed by 'watchful waiting' and; (e) the Preprostatectomy Study is examining the effects of short-term selenium supplementation on prostate cancer tissue in men with prostate cancer scheduled for a radical prostatectomy

associated with a high risk of the subsequent documentation of prostate cancer in an individual (Alsikafi et al. 2001; Fowler et al. 2001).

Prostatic intraepithelial neoplasia (PIN) is used to describe normal prostate glands and ducts that are lined with atypical cells. These cells have atypia in the nuclei as well as

cellular morphologic changes. The degree of change can be categorized into three grades. Grade 3 PIN is HGPIN and is the only grade of PIN which is associated with prostate cancer risk. The following evidence supports the association of HGPIN with prostate cancer: HGPIN incidence increases with age at a rate paralleling prostate cancer; HGPIN-like prostate cancer occurs more frequently in African-American men; prostate cancer is more likely to be found in prostates which contain HGPIN; and within the prostate HGPIN and cancer when found together are physically close to each other.

These findings support a conclusion that HGPIN is a premalignant lesion and hence identifies a group of men at significant risk of subsequently being diagnosed with prostate cancer (up to 50%). HGPIN is consequently a reasonable target for a chemoprevention trial. Such a study has been designed and is enrolling patients in a Southwest Oncology Group (SWOG) trial, SWOG 9917 (Marshall 2001). In this study, 466 men with HGPIN but no cancer on a biopsy will be enrolled and randomized to 200 mcg of L-selenomethionine or placebo orally daily for 3 years. If at any time with follow-up every 6 months for 3 years a digital rectal exam is suspicious for cancer or the PSA is elevated, a recommendation for a transrectal ultrasound-guided prostate biopsy is made. After the completion of 3 years of selenomethionine, every participant undergoes a prostate biopsy. The end point is the incidence of prostate cancer on biopsy.

*Selenium and Prostate Cancer Prevention in Men With Localized Prostate Cancer (The Watchful Waiting Study)*. The Watchful Waiting study, which is also NCI-sponsored, randomized 220 men with biopsy-proven prostate cancer who have elected not to undergo surgery, radiation, hormone therapy, or any other type of therapy, and are under the age of 85 (Stratton et al. 2003b). Treatment groups include 200 or 800 mcg of selenium per day or placebo. End points include PSA velocity, time to progression, time to treatment, alkaline phosphatase and chromagranin A levels. Statistical analyses performed in this study will be stratified by Gleason score (Fig. 5d).

*Selenium and Modulation of Biomarkers in Prostate Tissue (The Preprostatectomy Study)*. The Preprostatectomy Study is sponsored by the Department of Defense. This study enrolled men who were recently diagnosed with prostate cancer and were scheduled for a radical prostatectomy between three and six weeks from the time of enrollment (Stratton and Ahmann 2003). During that time, they are randomized to receive 200 or 400 mcg of selenium per day or placebo. In this study selenium levels in prostate tissue will be measured from the time of the original diagnostic biopsy and from the radical prostatectomy. This will determine whether selenium taken orally can affect selenium levels in prostate tissue. Tissue will also be analyzed for markers of cell growth and apoptosis using immunohistochemistry (Fig. 5e).

### 15.6.3 Soy Isoflavones

Epidemiologic studies have suggested that a diet rich in soy compounds may reduce the risk of prostate cancer (Cassidy 2003; Lee 2003b). Soybeans and other soy products contain isoflavones, which show promise as prostate cancer chemopreventive agents. Preclinical data suggest that soy isoflavones, such as genistein and diadzien, may play a role in the

hormonal regulation of prostate cancer by inhibiting the enzyme 5α-reductase (Barqawi et al. 2004). Early studies on soy isoflavones have also shown inhibition of cell growth and induction of apoptosis in prostate cancer cell lines. In a comparison of various dietary compounds used for chemoprevention, Agarwal and colleagues showed that the soy isoflavone, genistein, induced apoptosis in 30–40% of DU145 prostate cancer cells (Agarwal 2000). This study also provides evidence that genistein induces CDKI-mediated cell cycle arrest.

While this evidence sounds promising in favor of genistein as a prostate cancer chemopreventive agent, some studies have known DNA damage resulting from treating cells with this compound in vitro. DNA strand breaks were noted for high doses of genistein (100 µmol/L or more) in cultured cancer cells of mice and Chinese hamsters, as well as in human lymphoblastoid and blood lymphocyte cells (Miltyk et al. 2003). These data prompted Miltyk and colleagues to conduct a study on the genetic safety of soy isoflavones in men with prostate cancer. Their results indicated that participants taking as much as 600 mg per day never achieved a blood plasma genistein concentration above 27 µmol/L. DNA strand breaks in peripheral lymphocytes were measured by COMET assay, and no significant increase was found for any participant, suggesting that genistein does not lead to DNA damage within the dosages given in this study and may be safe to study in future clinical trials. However, this does raise a question regarding bioavailability of this agent.

Several studies on the use of soy isoflavones in prostate cancer prevention and treatment are underway. The H. Lee Moffit Cancer Center and Research Institute in Tampa, Florida, is currently conducting two studies, one on the effect of soy isoflavones versus placebo on hormone levels of stage II and III prostate cancer patients and the other on isoflavones versus lycopene in prostate cancer patients prior to radical prostatectomy. Phase II studies are also being conducted on patients with a negative biopsy and medial PSA levels (5–10 ng/ml) and cancer patients prior to radical prostatectomy, with an emphasis on apoptosis levels and cell proliferation rates. Examples of studies on soy isoflavones is presented in Table 15.4.

**Table 15.4.** Studies testing soy isoflavones for prostate cancer chemoprevention

| Research group | Phase | Population | Treatment |
|---|---|---|---|
| H. Lee Moffit Cancer Center | Pilot Study | Patients with stage I or II prostate cancer | Oral isoflavones twice daily and a multivitamin once daily vs. oral placebo twice daily and multivitamin once daily |
| H. Lee Moffit Cancer Center | Pilot Study | Stage I and II prostate cancer patients pre-prostatectomy | Oral isoflavones (3 dose groups) twice daily or oral lycopene (3 dose groups) twice daily or oral placebo twice daily |
| Cancer and Leukemia Group B | II | Patients who have an elevated PSA (5-10 ng/ml) and a negative biopsy | Oral soy protein once daily vs. oral placebo once daily |
| National Cancer Institute of Canada | II | Patients with high grade prostatic intraepithelial neoplasia | Combination of soy, vitamin E and selenium twice daily vs. placebo twice daily |
| Barbara Ann Karmanos Cancer Institute | II | Stage I and II prostate cancer patients pre-prostatectomy | One of three different dosage levels of soy isoflavones (amounts not specified) daily vs. placebo daily |

Clearly, soy isoflavones are of significant interest to the research community, and the results of these trials will provide important information regarding the efficacy of these compounds as a chemoprevention agent.

### 15.6.4 Cox-2 Inhibitors

COX-2 overexpression stimulates the production of prostanoids including PGE2, which induces angiogenesis in cancer tissue which thereby contributes to tumor blood supply (Banerjee et al. 2003). Prostate cancer cells containing high levels of COX-2 also display decreased levels of apoptosis (Fosslien 2001; Kirschenbaum et al. 2001). Inhibition of COX-2 in prostate cancer cell lines has been shown prevent PGE2-mediated expression of vascular endothelial growth factor (VEGF) (Liu et al. 2000) and induce apoptosis in prostate cancer cell lines (Godbey and Atala 2003). These data led to initiation of clinical studies testing effectiveness of COX-2 inhibitors for prostate cancer therapy and prevention. Currently, COX-2 research in prostate cancer falls into one of two categories, which include non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit both the COX-1 and -2 isoforms; and selective COX-2 inhibitors. The androgen inhibitor finasteride has also been shown to block COX-1 and -2 expression. Examples of compounds that have been under investigation are shown in Fig. 6.

Several COX-2 specific inhibitors are currently being tested in clinical trials, such as celecoxib (Celebrex) and Etodolac. Celecoxib has been shown to induce apoptosis in both PC-3 and LNCaP cell lines in a dose- and time-dependent manner (Hsu et al. 2000; Fosslien 2001). In addition, Pruthi and colleagues showed a decrease in prostate specific

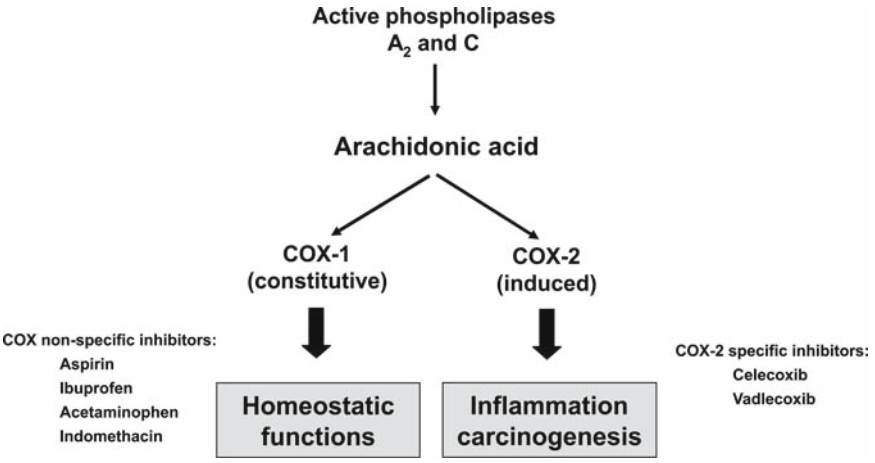


**Fig. 6 Isoforms of COX.** COX-1 is responsible for maintaining GI homeostatis and inhibition of this isoform is associated with GI toxicity. The COX-2 isoform is involved with the processes of inflammation and carcinogenesis and is a target for cancer chemoprevention. Numerous agents are in clinical development testing efficacy of COX-2 inhibition on carcinogenesis in a variety of tumor types

antigen (PSA) levels and doubling time in response to treatment with celecoxib in men who have recently undergone radiation therapy or radical prostatectomy (Pruthi et al. 2004). Johnson and colleagues conducted a study comparing several COX-2 specific inhibitors in vitro, including celecoxib (Johnson et al. 2001). Etodolac was investigated by Kamijo and colleagues in comparison to NS-398. Etodolac decreased cell proliferation in PC-3 and LNCaP prostate cell lines but not in normal prostate epithelial cells (Kamijo et al. 2001).

In vitro and preclinical animal data suggest that the COX-1 and -2 inhibitor, sulindac sulfone, exhibits antitumor activity against prostate cancer contributed to the development of several phase I/II clinical trials examining PSA response as well as measurable disease response rate as a single agent or in combination with taxotere. The secondary objectives include determination of time to disease progression and duration of response in patients with prostate carcinoma (NCI 2004a).

The COX-2 specific inhibitor, celecoxib, is also undergoing clinical development for prevention/treatment of prostate cancer. In one NCI-sponsored phase I/II study ongoing at the Johns Hopkins Oncology Center in Maryland, patients are randomized to receive either celecoxib or placebo prior to radical prostatectomy (NCI 2004a). The objectives of this study are: (1) to compare biomarker modulation (prostaglandin levels) in tissue samples of patients with localized prostate cancer treated with neoadjuvant celecoxib versus placebo followed by prostatectomy; (2) to compare the effect of these regimens on angiogenic factors within the prostate in these patients; and (3) to determine the pharmacokinetic and pharmacodynamic effects of celecoxib in these patients.

### 15.6.5 Milk Thistle

Milk thistle extract comes from the seed of the *Silybum marianum* plant and is used clinically for a variety of indications, including neural stimulation, immunostimulation (Wilasrusmee et al. 2002) and as a clinical treatment for certain liver diseases (Zi and Agarwal 1999; Singh et al. 2002). With respect to prostate cancer prevention, preclinical data on DU145 xenografts in nude mice suggests that silibinin (a primary component of milk thistle extract) inhibits cell growth in a dose- and time-dependent manner, and that excessive cell proliferation does not recur after the cessation of treatment. Recent studies have shown cell growth inhibition by milk thistle products occurs at the G1 phase (Shamberger and Frost 1969; Agarwal 2000; Dhanalakshmi et al. 2003) and provides a moderate induction of apoptosis in prostate cancer cell lines. This effect is particularly apparent when silibinin is coupled with other cytotoxic drugs (Tyagi et al. 2002).

Dhanalakshmi and colleagues observed silibinin combined with cytotoxic platinum compounds cisplatin and carboplatin for inhibition of cell growth in DU145 prostate cancer cell lines. They found that the combination of silibinin and carboplatin inhibited growth in 80–90% of cells, while the silibinin and cisplatin pairing caused 63–80% inhibition (Dhanalakshmi et al. 2003). These findings were significantly greater than the growth inhibition experienced by cells treated with either platinum compound alone (36–65% inhibition). Tyagi and colleagues conducted a similar study doxorubicin, and found that

growth inhibition occurred in 88% of DU145 cells treated with a combination of the drug and silibinin, as opposed to 41% in cells treated only with doxorubicin. This study also noted an increase in apoptosis with the combination treatment as opposed to either agent alone (Tyagi et al. 2002). These data indicate that silibinin may be used in conjunction with cytotoxic drug treatments to inhibit cell growth. Clinically, this is significant due to the danger of toxicity and negative side effects that may occur with both platinum-based treatments and doxorubicin.

Authors Zi and Agarwal chose a different course of study by looking at the effect of silibinin. They noted that intracellular PSA levels decreased upon treatment with silibinin in LNCaP prostate cancer cell lines (Zi et al. 2000). As a secondary endpoint to their original study on PSA, Zi and Agarwal also observed growth arrest in the G1 in cells treated with silibinin, but specified that this was not followed by increased apoptosis, which indicates that the ability of silibinin to induce cell death may be questionable when not combined with another substance (Zi and Agarwal 1999).

Overall, milk thistle byproducts appear to be more effective at reducing growth in prostate cancer cell lines, and may increase the effect of other chemopreventive drugs that are currently in clinical use.

### 15.6.6 Saw Palmetto

*Serenoa repens,* commonly known as saw palmetto, is often utilized as an herbal treatment option for men diagnosed with benign prostatic hyperplasia (BPH), the non-malignant enlargement of the prostate gland often exhibited in aging men typically associated with voiding symptoms resulting from increased pressure on the urethra (Knaus 2002). Despite the minimal clinical evidence of the therapeutic efficacy and safety of saw palmetto in the United States, the herbal supplement is frequently prescribed as an alternative therapy for BPH. The role of saw palmetto in cancer chemoprevention is speculative and the chemopreventive effects of saw palmetto will require extensive clinical and molecular based research.

An analysis of 18 clinical trials testing safety and efficacy of saw palmetto in patients with BPH showed some therapeutic benefit in urinary symptoms in patients with BPH. However there was considerable commentary on limitations in the analyses regarding the variation in clinical study design and duration of treatment (Wilt et al. 1998). Additional review of clinical studies conducted by Gerber and colleagues also implicated the significant positive effects of saw palmetto on urinary tract symptoms in BPH patients (Gerber 2000). Subsequently, Gerber and colleagues conducted a 6 month, randomized, placebo-controlled clinical trial that confirmed that saw palmetto was effective in reducing urinary tract symptoms in BPH (Gerber et al. 2001).

Some preliminary in vitro assessment of saw palmetto activity has been performed in prostate cancer cells. The primary therapeutic action is to inhibit 5-alpha reductase in forming DHT and, to a lesser extent, 3-alpha reductase, and to block the action of DHT to receptors on prostate cells via 3-ketosteroid reductase. Saw palmetto was shown to inhibit prostate tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA), demonstrating potential chemopreventive activity (Kapadia et al. 2002). Additional in vitro assessment of

saw palmetto in prostate cancer cell lines identified a correlation with saw palmetto and a decrease in COX-2 expression (Goldmann et al. 2001). In addition, Veltri and colleagues observed epithelial prostate cell chromatin restructuring following 6 months of saw palmetto treatment in a randomized placebo-controlled trial (Veltri et al. 2002). While some data suggest that saw palmetto shows promise as a prostate cancer-chemopreventive agent, extensive clinical studies will be required to test efficacy.

### 15.6.7 Resveratrol

Resveratrol is a polyphenolic compound abundant in grapes, red wine, and several types of nuts and berries. Considerable evidence suggests that resveratrol may have inhibitory effects in tumor proliferation, invasion and metastasis; and enhanced tumor cell apoptosis has been documented in studies targeting prostate, breast (Nakagawa et al. 2001; Scarlatti et al. 2003), colon (Wolter et al. 2001; Delmas et al. 2003), hepatic (Yu et al. 2003), and skin cancer (Niles et al. 2003). The seminal report by Pezzuto and colleagues showed that resveratrol elicits inhibitory action at each stage of carcinogenesis and that it has multiple targets including kinases, steroid hormone receptors and reactive oxygen species (Jang et al. 1997). In addition, resveratrol has been shown to impede activation of carcinogens by inhibition of phase I metabolic enzymes such as cytochrome P450 1A1 (Chun et al. 1999).

Studies in an in vivo rat model showed that resveratrol exerts potent anti-inflammatory effects by inhibition of COX-1 and -2 (Jang et al. 1997; Subbaramaiah et al. 1998). It is of interest that inhibition of both COX isoforms is by direct interaction with the enzyme in addition to blocking transcription of COX-2 by inhibition of NFκB transactivation activity. Also relevant to resveratrol's profile as a cancer-chemopreventive agent is its capacity to induce cell cycle arrest in G1, and to trigger caspase-dependent, p53-mediated and bcl-2-sensitive apoptotic responses (Stewart et al. 2000). Other in vitro studies showed that resveratrol differentially inhibits members of the protein kinase C family (Stewart et al. 1999; Slater et al. 2003). Perhaps the most important mechanism elicited by resveratrol on prostate cancer cells is its ability to modulate the androgen receptor. Mitchell and colleagues showed that resveratrol exerts antiandrogenic effects on the hormone-responsive prostate cancer cell line, LNCaP (Mitchell et al. 1999). The inhibitory mechanism involves inhibition of expression of the androgen receptor, the androgen receptor co-activator ARA70 and androgen-mediated genes, including PSA. These data suggest that resveratrol may be a prime candidate for prostate cancer chemoprevention and as a neoadjuvant therapy for prostate cancer.

## 15.7 Conclusion

Prostate cancer is a major health problem and is the most commonly diagnosed cancer in North American males. Although incidence has increased in the past decade, probably due to improved screening, mortality has decreased because disease is diagnosed at an earlier

**Table 15.5** Examples of prostate cancer prevention trials sponsored by the National Cancer Institute (NCI 2004)

| Title | Primary Endpoint(s) |
|---|---|
| Phase III randomized study of selenium and vitamin E for the prevention of prostate cancer (SELECT) | Compare the effect of selenium and vitamin E administered alone vs in combination on the clinical incidence of prostate cancer. |
| Phase III randomized study of selenium as chemoprevention of prostate cancer in patients with HGPIN | Compare the effects of selenium versus placebo on the 3-year incidence rate of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. |
| Phase II Randomized Study of Dietary Soy in Patients With Elevated PSA Levels | Compare the reduction in the rate of prostatic cellular proliferation in patients with an elevated PSA (5 to 10 ng/mL) and a negative biopsy for prostate cancer when treated with daily soy protein supplements vs placebo.<br>Compare the effect of these regimens on additional biomarkers of prostate cancer (PSA, high-grade prostate intraepithelial neoplasia, induction of apoptosis, sex steroid receptor expression, and loss of glutathione S-transferase-pi) in these patients. |
| Phase II Randomized Study of Vitamin E, Selenium, and Soy Protein Isolate in Patients with High-Grade Prostatic Intraepithelial Neoplasia | Determine whether nutritional supplementation with soy protein isolate, vitamin E, and selenium can delay the time to development of invasive prostate cancer (disease-free survival) in patients with high-grade prostatic intraepithelial neoplasia.<br>Determine the effect of this supplementation on intermediate endpoints that may reflect a lessened risk of invasive prostate cancer (e.g., serum PSA levels, hormone levels, lycopene, malondialdehyde, vitamin E, and reduced thiol groups) in these patients. |
| Phase II Randomized Study of Vitamin E, Selenium, and Soy Protein Isolate in Patients with High-Grade Prostatic Intraepithelial Neoplasia | Determine whether nutritional supplementation with soy protein isolate, vitamin E, and selenium can delay the time to development of invasive prostate cancer (disease-free survival) in patients with high-grade prostatic intraepithelial neoplasia.<br>Determine the effect of this supplementation on intermediate endpoints that may reflect a lessened risk of invasive prostate cancer (e.g., serum PSA levels, hormone levels, lycopene, malondialdehyde, vitamin E, and reduced thiol groups) in these patients. |
| A Chemoprevention Study of an Investigational Drug in Men with High Grade Prostate Intraepithelial Neoplasia (PIN) | This is a study of an investigational medication that may reduce high grade PIN and prevent the occurrence of prostate cancer. |
| Phase II Randomized Study of Toremifene Followed by Radical Prostatectomy in Patients with Stage I or II Adenocarcinoma of the Prostate | Compare the percent of high-grade prostatic intraepithelial neoplasia (HGPIN) present in the radical prostatectomy tissue (excluding the luminal area) of patients with stage I or II adenocarcinoma of the prostate treated with toremifene vs observation alone followed by radical prostatectomy.<br>Compare the absolute and relative changes in HGPIN in patients treated with toremifene vs observation alone. |

(continued)

**Table 15.5** (continued)

| Title | Primary Endpoint(s) |
|---|---|
| Phase IIB Randomized Chemoprevention Study of DFMO in Patients at High Genetic Risk for Prostate Cancer | Compare the levels of polyamines (putrescine, spermidine, and spermine) and progression-related genes in the prostate tissue of patients at high genetic risk for prostate cancer treated with eflornithine (DFMO) vs placebo. |
| Phase I Study of Lycopene for the Chemoprevention of Prostate Cancer | Determine any dose limiting toxicities and the maximum tolerated dose of lycopene administered orally as a food based delivery system in healthy male subjects 18–45 years of age for the chemoprevention of prostate cancer. |
| Evaluation of risk factors which predict the transformation of early stage to clinically aggressive prostate cancer | Evaluate risk factors which predict the transformation of early stage to clinically aggressive disease. |
| Randomized Pilot Study of Isoflavones versus Lycopene Prior to Radical Prostatectomy in Patients with Localized Prostate Cancer | Compare the effect of isoflavones vs lycopene prior to radical prostatectomy on intermediate biomarkers (e.g., indices of cell proliferation and apoptosis) in patients with localized prostate cancer.<br>Compare the effects of these nutritional supplements on increases in plasma levels and tissue levels of these agents in these patients. |

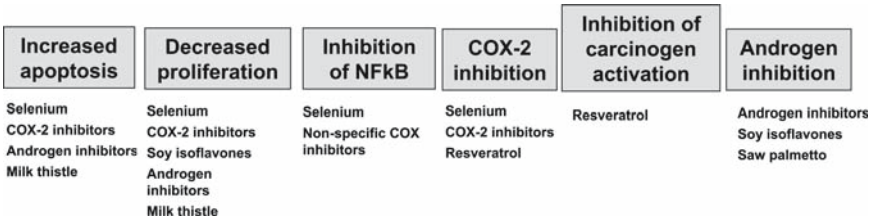


**Fig. 7 Mechanisms of inhibition of carcinogenesis.** Successful cancer preventive agents may have a variety of molecular mechanisms including increased apoptosis, decreased proliferation, inhibition of proteins that regulate inflammation, inhibition of activation of carcinogens and androgen inhibition. Agents currently in preclinical and clinical development for prostate cancer prevention elicit more than one of these mechanisms on prostate cancer cells

stage and treatment strategies have improved. Prevention of prostate cancer and impediment of disease progression at an early stage would have a significant impact on healthcare for men and healthcare economics. The paradigm of prostate cancer prevention is shifting from primary prevention to treatment of the process of carcinogenesis. Successful prostate cancer prevention strategies will be achieved not only by preclusion, inhibition of reversal of early stages of carcinogenesis that lead to tumor development, but also by eradication of incipient populations of more aggressive malignant cells. The latter strategy, which includes immune surveillance, would arrest the process of carcinogenesis prior to development of clinically significant neoplastic disease.

The significant advances in molecular biology techniques including microarray and proteomics within the past decade are allowing us to now identify potential surrogate endpoint biomarkers for prostate cancer prevention trials and recognize risk categories for development of prostate cancer. Currently, several agents in clinical development show promise for effective prostate cancer prevention and numerous clinical studies are ongoing (Table 15.5). In the majority of ongoing studies, development of biopsy-proven prostate cancer is the primary endpoint. Validation of early markers of carcinogenesis will have a profound impact of chemoprevention studies with regards to time and costs.

Several molecular chemopreventive mechanisms have been proposed for each agent being studied (Fig. 7). These include inhibition of apoptosis, induction of cell cycle arrest, blockage of androgen activity, inhibition of COX-2 and inhibition of activation of carcinogens. While many agents are in preclinical development and clinical trials, none have been proven to be effective. Further study of potential mechanisms of action will help development of more targeted preventive agents.

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# Cervical Cancer Prevention

# 16

F. Garcia, T. Nuno, A.L. Mitchell

## 16.1 Incidence and Mortality of Cervical Cancer

Cervical cancer is the most common gynecologic malignancy worldwide, accounting for about 500,000 new cases each year (WHO Report 2006). Most of these cases (90%) occur in the developing world where it is the second most common malignancy in women after breast cancer (WHO Report 2006). By contrast, in the United States (U.S.) cervical cancer has decreased dramatically since the introduction of cytologic screening (Pap smear), and is now a relatively infrequent neoplasm, especially among well-screened majority populations with access to health care services. In the U.S., an individual woman's lifetime risk of developing cervical cancer is estimated to be 1 in 142 (Ries et al. 2007). In 2008, it is estimated that there were approximately 11,070 new cases of cervical cancer, and 3,870 deaths in the U.S. (Jemal et al. 2008).

In the developed world, cervical cancer disproportionately affects poor and minority women without adequate access to cervical cancer screening. It is estimated that nearly 50% of U.S. cervical cancer cases occur in women who have never been screened, and an additional 10% in women who have not been screened within five years of their diagnosis (Sawaya et al. 1999; Leyden et al. 2005; Schwartz et al. 1996). Significant racial and ethnic disparities exist with regard to screening, incidence, mortality, and survival associated with the diagnosis of cervical cancer in this country (Table 16.1) (2003a). Notably, the gap in incidence and mortality between White women and other racial/ethnic groups increases with age (2001). Although disparities in incidence and mortality have decreased in recent years, cervical cancer incidence remains about 50% higher among African American women (11.4 out of every 100,000) compared to White women (8.5 out of every 100,000) (Ries et al. 2007), and cervical cancer mortality among African American women which is the highest (4.9 out of every 100,000) of any racial or ethnic group (Ries et al. 2007). African American women

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| | White | Black | Hispanic[a] |
|---|---|---|---|
| Incidence (per 100,000) | 9.2 | 12.4 | 16.8 |
| Mortality (per 100,000) | 2.7 | 5.9 | 3.7 |
| 5-year survival (%) | 72.9 | 61.0 | |

**Table 16.1** Racial/ethnic disparities in cervical cancer

[a]The category Hispanic is not mutually exclusive from Black or White

are less likely to present with localized disease (44% compared to 56% of White women) and are twice as likely to die of their disease (Ries 1999). Additionally, African American women are less likely to receive surgery (33.5% compared to 48.2% in White women) and more likely to receive radiation (35.3% compared to 25.2%) (Howell et al. 1999a). Women of Vietnamese origin have the highest age-adjusted incidence rate (43 out of every 100,000) and Japanese origin individuals have the lowest (5.8 out of every 100,000) (Miller 1996). Surveillance, epidemiology and end results (SEER) data for 2000 through 2003 continue to demonstrate a disproportionately higher incidence (14.2 out of every 100,000) and mortality (7.3 out of every 100,000) for Hispanic compared to non-Hispanic whites (3.4 and 2.3 out of every 100,000 for incidence and mortality rates, respectively) (American Cancer Society 2006). Hispanics are also more likely to present with advanced stages of invasive disease (Napoles-Springer et al. 1996; Mitchell and McCormack 1997; Howe et al. 1998). Not surprisingly, higher rates of precursor lesions have been documented for both African American and Hispanic women (Howe et al. 1998; Liu et al. 1998).

## 16.2 Etiology of Cervical Cancer

Prior to the definitive identification of human papillomavirus (HPV) infection as a necessary agent in cervical carcinogenesis, observational studies had already suggested that a sexually transmitted agent was involved in the disease and that male sexual behavior could affected the risk of cancer in the female partners. Specifically, research demonstrated geographic clustering of cervical and penile cancers, the low prevalence of disease among non-sexually active women, and increased risk in partners of men whose first wives had died of cervical cancer. More sophisticated and recent molecular epidemiology work has further clarified this relationship (Buckley et al. 1981; Zunzunegui et al. 1986; Agarwal et al. 1993; Thomas et al. 1996; Castellsague et al. 2002).

In the mid 1970s, Zur Hausen first suggested a relationship between HPV infection and cervical cancer (zur Hausen 1977); by the early 1980s, electron-micrography work had identified the presence of the virus in cervical intraepithelial neoplasia (CIN) (Meisels et al. 1983). Since that time, cervical carcinoma and its precursor lesion, CIN, have been consistently causally linked to the sexual transmission of HPV infection (Schiffman et al. 1993; Morris et al. 1996).

HPV infection is ubiquitous and widespread across many species; more than one hundred different types can infect humans. Transmission of this small icosohedral double-

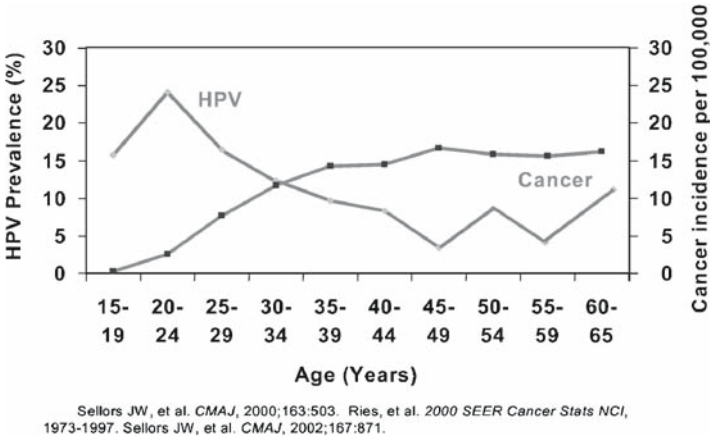


**Fig. 1** HPV point prevalence and cervical cancer incidence by age

stranded DNA virus occurs through direct contact with epithelial surfaces. Lower genital tract HPV infection is common during the second and third decade of life, and is a marker of human sexual activity (Fig. 1). The vast majority of immunocompetent women appear to clear the infection without sequelae. By comparison, cervical cancer is a relatively rare event that peaks in the fourth and fifth decade. Infections with HPV are classified as high-risk (oncogenic) or low-risk (non-oncogenic) genotypes, based on the association with cervical cancer. High-risk HPV infection with types 16 and 18 has the highest prevalence and is associated with approximately three quarters of all cancer and high-grade precursor lesions. Additional high-risk types include 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, and possibly 26, 53, and 66. By contrast, HPV 6 and 11, the etiologic agents of genital warts, and types 42, 43, 44, 54, 61, 70, 72, and 81 are infrequently associated with advanced cervical lesions and are considered low-risk (Palefsky and Holly 1995; Franco 1996; Munoz et al. 2003). Beyond viral type differences, there are significant differences in genetic variation, viral load (van Duin et al. 2002), and persistence which may confer increased risk for cervical neoplasia. In total, the strength of the association between high-risk HPV infection and cervical cancer is such that very nearly all squamous cell cervical malignancies are now thought to be related to HPV infection. It is clear that HPV infection is a necessary prerequisite, but by itself an insufficient cause, for cervical cancer.

## 16.3 Natural History of Cervical Cancer

HPV enters the basal layer of the genital tract through micro-tears that occur in the squamous epithelium during the course of sexual activity. The mitotically active transformation zone is particularly vulnerable, especially early in adolescence when it covers a relatively broad surface area of the cervix. The viral DNA enters the cellular nucleus where

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it exists as a circular episome composed of three distinct regions. In general, the long control region (LCR) regulates early viral transcription, while the late (L) region encodes structural proteins involved in the assembly and production of the viral capsid. The early (E) region encodes proteins necessary for viral replication. The E6 and E7 gene products bind p53 and retinoblastoma proteins respectively, and interfere with tumor suppressor functions leading ultimately to cellular transformation (Shirodkar et al. 1992; Scheffner et al. 1993; Jones et al. 1997; Denk et al. 2001). Precursor lesions and cervical carcinoma require the non-random integration of extra chromosomal viral DNA into the host genome (Thorland et al. 2000). This integration of viral DNA into the host genome results in disruption of the E2 region of the viral episome (Choo et al. 1987), which is responsible for down regulation of E6 and E7, and ultimately to cellular transformation.

As the cells of the basal layer of the squamous epithelium mature, they are pushed out to the epithelial surface where replication of the viral genome takes place. Eventually the viral DNA copies are packaged in the protein capsid. These viral particles are released through the normal process of epithelial desquamation and cell death, and upon shedding may contribute to transmission.

Generally, HPV infection is transient, asymptomatic, clinically insignificant, and occasionally associated with temporary cytologic and/or histologic abnormalities (Fig. 2). The median duration of infection has been estimated to be about eight months for young sexually active women (Ho et al. 1998). This time period may be longer for women infected with oncogenic viral types (Richardson et al. 2003). Although older individuals may be less likely to clear the virus, in general, about 90% of infected women will have undetectable evidence of infection at 24 months (Moscicki et al. 1998).

In some cases, infection leads to characteristic cellular abnormalities beginning at the basal layer and involving increasingly more layers of cervical epithelium. These are described histologically as CIN (Wright 1994). CIN is generally divided into low-grade (CIN 1) and high-grade (CIN 2 and CIN 3) disease. This grading generally reflects the underlying risk for progression to malignancy (highest for CIN 3) and decreasing likelihood of spontaneous regression (highest for CIN 1) (Ostor 1993; Barker et al. 2001). Low-grade CIN occurs most frequently in younger women (Jones and Novis 2000) and is more likely

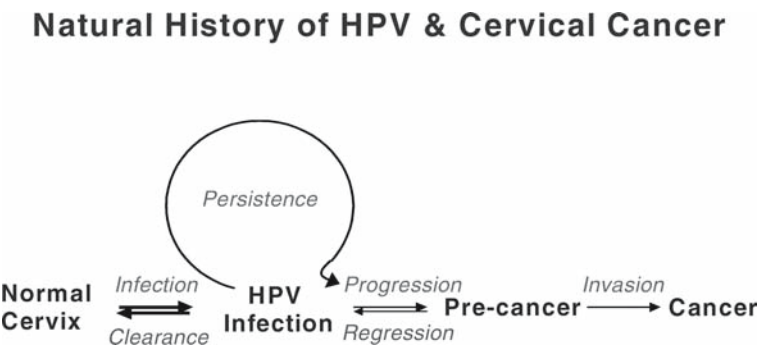


**Fig. 2** Natural history of HPV and cervical cancer, adapted (Schiffman and Kjaer 2003)

to spontaneously regress in that age group. Viral persistence is a prerequisite for progression from high-grade CIN and invasive carcinoma, and is related not only to viral type, but also to viral load and increasing age (Koutsky et al. 1992; Londesborough et al. 1996; Ho et al. 1998). The protracted process of progression (estimated at more than 10 years) from CIN 3 to invasive disease provides an ideal time frame that permits screening, identification and treatment of these precursor lesions.

## 16.4 Co-Factors for Cervical Cancer

The largely endemic nature of HPV infection and the relative rarity of cervical cancer argue for an important role of co-factors that may affect progression or regression of cervical cancer precursors (Table 16.2). Historically, parity has been identified consistently as an important risk factor in cervical cancer (Brinton et al. 1989; Parkin et al. 1994; Yoo et al. 1997; Munoz et al. 2002). It is unlikely that the effect of parity is simply related to childbirth itself; instead parity may be a surrogate for a spectrum of sexual behavior including total lifetime number of male sexual partners, early age at sexual intercourse, co-morbid sexually transmitted infections (STI), and potentially even smoking and hormonal contraceptive use. With regard to smoking, nicotine metabolites can be recovered in the cervical and vaginal secretions of women who smoke or those exposed passively (McCann et al. 1992; Prokopczyk et al. 1997). In particular, the use of tobacco products may be associated with a two- to fourfold increased risk for cervical cancer and its precursors (McCann et al. 1992; de Vet et al. 1994; Prokopczyk et al. 1997; Deacon et al. 2000). Likewise, there may be a role for other STIs that infect the female lower genital tract,

**Table 16.2** Theoretical framework for cervical cancer prevention

| Primary Prevention – HPV infection prevention | Secondary Prevention – CIN detection and treatment | Tertiary Prevention – Cervical cancer treatment and control |
|---|---|---|
| **Behavioral modification** | **Behavioral modification** | **Behavioral modification** |
| • Sexual | • Sexual | • Tobacco cessation |
| • Tobacco cessation | • Tobacco cessation | |
| • Prophylactic vaccine | • Screening programs | **Medical therapeutics** |
| | | • Radical surgery |
| **Nutrition** | **Medical therapeutics** | • Radiation therapy |
| | • Excisional therapy | • Chemotherapy |
| | • Therapeutic vaccines | • Therapeutic vaccines |
| | • Chemopreventive agents | |
| | | **Surveillance** |

and may potentiate the oncogenicity of high-risk HPV. This may be mediated through the cellular-mediated immune mechanism (Konya and Dillner 2001) or a localized inflammatory effect on the cervico-vaginal epithelium (van Duin et al. 2002; Garcia et al. 2003b). Long-term use of combined oral contraceptive pills (OCPs) is also associated with cervical cancer risk in the presence of HPV infection (Moreno et al. 2002). These findings are particularly difficult to interpret given the complex nature of sexual behavior and the adverse effect of multiparity on the risk for cervical cancer. It is possible that the effects of tobacco and OCP use are mediated by promoting persistence of the viral infection.

**Table 16.2** Risk Factors Associated with HPV Infection, Cervical Intraepithelial Neoplasia, and Cancer

- Smoking
- Lower genital tract infection (chlamydia, herpes simplex virus [HSV], bacterial vaginosis)
- Sexual behavior (number of partners, age at first intercourse, partner's sexual behavior)
- Parity
- Impaired immune function (HIV/AIDS, post transplantation, chronic steroid, collagen vascular disease, pregnancy)
- Hormonal combined oral contraceptive use
- Nutrition
- Poverty (low socio-economic status)
- Lack of access of healthcare/screening

## 16.5 Viral Persistence

Numerous studies consistently demonstrate that persistent, as compared to transient, high-risk HPV infection is required for the development of cervical cancer precursor lesions (Koutsky et al. 1992; Ho et al. 1995; Coker and Bond 1999; Hernandez and Goodman 1999; ter Harmsel et al. 1999). It has also been shown that persistent high risk HPV infection is a prerequisite for development and maintenance of CIN 3 (Meijer et al. 1999), while low-risk type infections have very high rates of regression (Moscicki et al. 1998).

Clearance of HPV from the genital tract requires an active cell-mediated immune response. Cellular immune response is characterized by an interaction between antigen presenting cells (APCs), T-helper cells, and cytotoxic T cells. Activated natural killer (NK) cells may also play a role. Cell-mediated viral clearance and control of tumor growth were identified in studies of CIN in human immunodeficiency virus (HIV)-positive women. The observation that CIN occurs with disproportionate frequency among immunocompromised women (e.g., acquired immunodeficiency syndrome [AIDS], transplantion), suggests that CD4 lymphocytes (T-helper cells) are involved in prevention or limitation of HPV-associated lesions (Maiman 1998). Studies of immunocyte counts in HPV lesions

have confirmed the role of the cellular immune system in controlling viral infection. Significantly, greater numbers of T-lymphocytes and macrophages are found within the stroma and epithelium of HPV lesions that regress as compared to non-regressing lesions (Coleman et al. 1994).

Cytotoxic responses against HPV are mediated by T helper cells (CD4 cells) and antigen presenting cells such as Langerhans cells (LCs), whose interactions result in the stimulation of cytotoxic T cells (CD8 cells). Immune response is generated and maintained through the release of cytokines, and intracellular signals, from these different cell types. Cytokines secreted by CD4 cells include type 1 cytokines such as interleukin 2 (IL-2) and interferon gamma (IFN), and type 2 cytokines, including IL-4, IL-6, and IL-10. Type 1 cytokines are immuno-stimulatory for cell-mediated immune response; they promote CD8 responses, activate NK cell functions, and have been shown to be capable of limiting tumor growth. Interleukin 12 (IL-12) is a type 1 cytokine secreted by dendritic cells, including Langerhans' cells (LCs), and has been demonstrated to induce differentiation of naïve T cells, to up-regulate IFN gamma production in T cells and NK cells, leading to anti-tumor activity (Nastala et al. 1994; Zola et al. 1995; Clerici et al. 1998; Giannini et al. 1998).

Cytokine expression in cervical tissue has been variously associated with cervical intraepithelial neoplasia. Investigators have found a significant increase in the density of IL-4 positive cells in low grade and high grade squamous intraepithelial lesions (LSILs and HSILs), compared with histologically normal tissues from adjacent ectocervical regions (al-Saleh et al. 1995). Another study showed that expression of IL-10 increased continuously from a relatively low level in normal ectocervix to a high level in HSIL, and that IL-12 expression was higher in LSIL than in HSIL (Giannini et al. 1998). In women with CIN 3, in vitro production of IL-2 by peripheral mononuclear blood cells (PMBCs) was found to be decreased, while production of type 2 cytokines IL-4 and IL-10 increased in women with more extensive HPV infection (Clerici et al. 1997).

## 16.6 Screening and Early Detection of Cervical Cancer

### 16.6.1 Cytologic Screening

Exfoliative cytology has been the mainstay of cervical cancer prevention since its description by Papanicolau in the 1940s, and its large-scale adoption is frequently credited for the drop in cervical cancer incidence and mortality in North America and Western Europe (Anttila et al. 1999). This technique takes advantage of the prolonged pre-invasive nature of CIN, by repetitively sampling the transformation zone of the uterine cervix where the process of metaplasia turns the columnar epithelium of the endocervical canal into the more robust mature squamous epithelium of the ectocervix and vagina. It is these metaplastic cells that are the most vulnerable to oncogenic HPV infection and most invasive and precursor lesions arise from this area. Detailed evidence-based screening recommendations

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**Table 16.3** Summary of American Cancer Society (ACS) recommendations for human papillomavirus (HPV) vaccine use to prevent cervical cancer and Its precursors (reprinted with permission from Saslow et al. 2007)

| |
|---|
| Routine HPV vaccination is recommended for females aged 11 to 12 years: |
| ○ Females as young as age 9 years may receive HPV vaccination |
| ○ HPV vaccination is also recommended for females aged 13–18 years to catch up missed vaccine or complete the vaccination series |
| ○ There are currently insufficient data to recommend for or against universal vaccination of females aged 19–26 years in the general population. A decision about whether a woman aged 19–26 years should receive the vaccine should be based on an informed discussion between the woman and her health care provider regarding her risk of previous |
| ○ HPV exposure and potential benefit from vaccination. Ideally the vaccine should be administered prior to potential exposure to genital HPV through sexual intercourse because the potential benefit is likely to diminish with increasing number of lifetime sexual partners |
| ○ HPV vaccination is not currently recommended for women over age 26 years or for males |
| ○ Screening for cervical intraepithelial neoplasia and cancer should continue in both vaccinated and unvaccinated women according to current ACS early detection guidelines |

and triage algorithms for the management of abnormal cervical cytology are published elsewhere (Saslow et al. 2002; Wright et al. 2002, 2003b).

Although in widespread use there have never been, nor would it now be ethical to conduct, large randomized control trials of cervical cytology. Nonetheless, prolonged clinical experience with this technique has permitted the characterization of the test performance qualities. The sensitivity of cytologic screening is estimated to range between 50 and 70%, with specificity in the 70% range (Fahey et al. 1995, 1999).

More recent technologic advances in cytologic processing and technology aim to improve the performance characteristics of this screening technique. One important advance has been improvement of the quality of the specimen submitted for analysis, with thin-layer cytologic slides prepared from specimens collected in a liquid fixative. This technique appears to reduce false positive rates by improving the interpretability of the slide, by decreasing the presence of cells and debris associated with bleeding and inflammation. The thin layer also slightly improves the sensitivity of cytology (Lee et al. 1997; Hutchinson et al. 1999; Vassilakos et al. 1999; Belinson et al. 2001; Clavel et al. 2001) by providing a more evenly distributed cellular sampling which may lead to more accurate interpretation. The sum of the evidence is sufficiently compelling to recommend that with the use of thin layer cytology cervical cancer screening intervals may be lengthened to every two years (after three consecutive normal screens) in women at least 30 years of age (Saslow et al. 2002).

A variety of computerized cytologic screening devices have entered routine clinical use. The automated image-analysis, algorithm-based screening devices generate a score indicative of the risk for significant disease and identifies high-risk fields for pathologic review (Patten et al. 1997; Howell et al. 1999b). Such devices have made their way into large laboratories where they are used largely to comply with federally mandated re-screening of 10% of negative cytologies, and increasingly for primary screening. Such technology

**Table 16.4** Published chemopreventive clinical trials for cervical intraepithelial neoplasia (CIN)

| Agent | Published trials | Spectrum of CIN | References |
|---|---|---|---|
| Retinoids | | | |
| Retinyl acetate topical gel | 1 | 1–2 | Romney et al. (1985) |
| All-*trans* retinoic acid topical[a] | 7 | 1–3 | Surwit et al. (1982), Meyskens et al. (1983, 1994), Graham et al. (1986), and Weiner et al. (1986) |
| 4-HPR | 1 | 2–3 | Follen et al. (2001) |
| 9-*cis* retinoic acid | 1 | 2–3 | Alvarez et al. (2003) |
| Micro-nutrients | | | |
| Beta carotene | 6 | 1–3 | de Vet et al. (1991), Fairley et al. (1996), Manetta et al. (1996), Romney et al. (1997), Mackerras et al. (1999), and Keefe et al. (2001) |
| Folate | 3 | 1–2 | Butterworth et al. (1992a,b) and Childers et al. (1995) |
| Vitamin C | 2 | 1–2 | Romney et al. (1987) and Mackerras et al. (1999) |
| Diflouromethylornithine(DFMO) | 1 | 3 | Garcia (1998a,b) |
| Indole 3-carbinole[a] | 2–3 | 2–3 | Bell et al. (2000) |

[a] Positive phase 2 trial results

specifically addresses the contribution of human misclassification to cytologic inaccuracy, which results from fatigue and human error.

### 16.6.2 HPV Testing

The etiologic role of HPV in cervical cancer has lead to incorporation of HPV testing into a variety of screening and management algorithms for cervical disease. Oncogenic HPV testing has been proposed as a primary screening modality for screening for cervical cancer and its precursors (Cuzick et al. 2003). While in some settings HPV testing is more sensitive than cervical cytology, the endemic nature of high-risk oncogenic HPV infection, especially among young reproductive age populations, would lead to low specificity of screening with predictably high false positive rates (see Fig. 1) (Cuzick et al. 2000).

More recent guidelines have opened the door to the use of HPV testing as an adjunct to cytology, in an effort to safely extend screening intervals (Fig. 3) (Saslow et al. 2002;

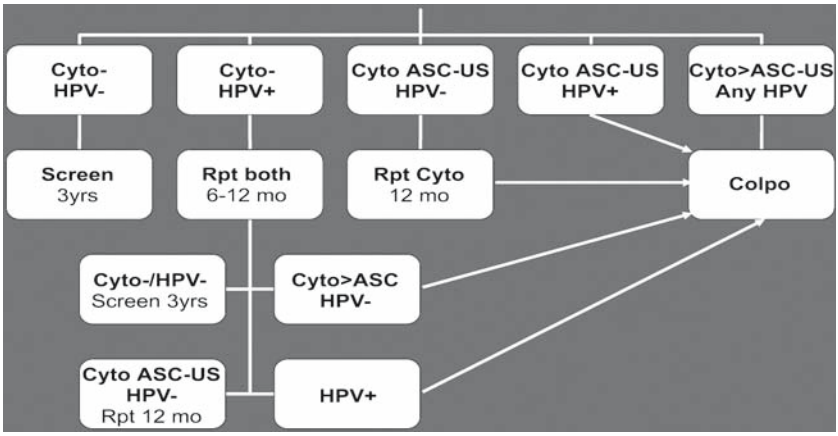


**Fig. 3** Management of Cytology/HPV Results

Wright et al. 2004). In general, the test performance characteristics of combined HPV and cytology screening (sensitivity of about 92–100% and specificity of 70–96%) exceed that of cytology alone (Wright et al. 2004). Current evidence suggests that co-testing with HPV and cytology, if it is performed, should be limited to women 30 years of age and older and should be performed no more frequently than every three years (Wright et al. 2004).

High-risk HPV testing has come into clinical use as one of three evidence based management strategies for the triage of atypical squamous cells of undetermined significance (ASCUS) cytology (along with repeat cytology and colposcopy). The sensitivity of HPV DNA hybrid capture testing for the detection of CIN 2 and CIN 3 is estimated to be between 80 and 100% (Wright et al. 1998; Sherman et al. 2002); women with negative testing in this setting have an extremely low likelihood of having clinically significant disease. When testing is available from liquid-based cytology specimen or when viral specimens are co-collected at the time of screening cytology, this is the preferred triage for the ASCUS pap (Wright et al. 2002).

## 16.7 Therapeutic Approach to Precursor Lesions of Cervical Cancer

The foundation of cervical cancer precursor therapy involves access to definitive colposcopic evaluation and histologic diagnosis. Treatment has been divided into ablative and excisional modalities. Excisional therapy has generally been reserved for high-grade lesions (CIN 2 and 3) with true malignant potential. Loop electrosurgical excision procedure (LEEP) requires an experienced operator but may be performed in the office setting. It is the most common approach to treatment of high-grade lesions in the U.S., is well tolerated by patients, and results in a histologic specimen that is evaluable for evidence of invasion. Bleeding and infection are potential complications of LEEP procedures

and may occur in about 2% of cases (compared to 10% for cold knife conizations) (Montz 2000). While cervical stenosis and impaired fertility are rare complications, women who undergo LEEP may be at increased risk for pre-term delivery and low birth weight infants in subsequent pregnancies (Samson 2005).

Historically, cryotherapy was used most commonly for low-grade lesions (CIN 1), which should be treated only when they are persistent (24 months or more) (Wright et al. 2003). Ninety percent (90%) of these lesions will regress spontaneously within 2 years (Ostor 1993). The regression rate is generally lower for women after their mid-thirties and among those who smoke. Cryotherapy is safe, effective, and economical; typically requiring less operator experience than excisional modalities. It does not, however, produce a histologic specimen and should never be used in the presence of endocervical diseases or when there is any question of potential microinvasive disease. In general the resolution rates for both these therapies is in the 90% range (Martin-Hirsch et al. 2000). A randomized trial of cryotherapy, laser vaporization, and LEEP found comparable cure rates for CIN 2/3 (Mitchell et al. 1998a). This trial also found that failure rates were highest for women with large lesion (three or four quadrants). Previously treated patients, those over 30 years of age, and women with oncogenic HPV infection are at significantly higher risk for treatment failure. Outpatient therapeutic modalities may lend themselves well for single visit and "see-and-treat" therapy that obviate the need for biopsy and even colposcopic examination, and may promote compliance among poor or underserved women.

## 16.8 Novel Agents for Cervical Cancer Prevention

### 16.8.1 Chemopreventive Agents

The search for chemopreventive agents for use in cervical cancer prevention is logical given the well characterized, protracted preinvasive character of CIN, encouraging preclinical data, and epidemiologic findings that suggest a protective role for a variety of nutritional agents. These chemoprevention studies, however, have been largely disappointing and plagued by a variety of methodological challenges. Problems include a lack of consensus as to the appropriate grade of disease to be studied (CIN 1, 2 or 3), the appropriate definition of response (e.g., histologic regression, viral clearance) given significant rates of spontaneous regression, the selection of appropriate endpoint biomarkers, as well as ethical and safety considerations (withholding treatment for potentially preinvasive disease). Despite these challenges, a significant number of trials have been conducted in this area (Table 16.4). A thorough review of cervical chemoprevention trials was published by Mitchell and colleagues (Follen et al. 2003).

Topical and oral retinoids are a group of agents that have been well characterized and that have significant promise in this tissue type. These agents may decrease the expression of E6/E7, tumor growth factors, and telomerase activity associated with carcinogenesis (Sizemore et al. 1998; Ding et al. 2002). They have generally good toxicity and tolerability

profiles and early phase I data suggest significant chemopreventive activity (Surwit et al. 1982; Meyskens et al. 1983; Weiner et al. 1986). One clinical trial of cervical all-*trans* retinoic (0.327%) acid application demonstrated significant activity for CIN 2 (43% compared to 27% in the placebo group), but not for CIN 3 (Meyskens et al. 1994). Likewise, trials of an oral synthetic retinoid (4-HPR) and *cis* retinoic acid have been negative (Follen et al. 2001; Alvarez et al. 2003). Indole-3-carbinol is another promising agent that may in part explain the cancer protective effect of cruciferous vegetable intake in some epidemiologic studies. This compound has been demonstrated to induce the protective effective cytochrome p-450 (Wattenberg and Loub 1978; Grubbs et al. 1995). A phase II study, of two different doses of this agent for 90 days in subjects with CIN 2/3, reported a consistent response rate of 44–50% (compared to no responders in the control group) (Bell et al. 2000). Randomized trials of imiquimod, a topical cellular immune response modulator, used for HPV-related genital condyloma, have been performed although not yet reported for cervical disease endpoints. Clinical trials are ongoing at the Arizona Cancer Center for selective cyclooxygenase-2 (COX-2) inhibitors (e.g., celecoxib) and green tea related compounds (e.g., polyphenon E), and are planned for diindoylmethane (e.g., DIM).

### 16.8.2 Therapeutic Vaccines

An alternative therapeutic approach has targeted the elimination of high-grade precursor and even invasive lesions in individuals with established HPV infection. Such a strategy is dependent on cellular immune response, rather than antibody development. In general, this involves the stimulation of a cytotoxic T-lymphocyte response to the E6 and E7 oncoproteins. HPV peptide trials that have used this approach have shown modest promise. A small trial of a preparation using E7 epitope peptides in subjects with cervical and vulvar squamous intraepithelial lesions demonstrated T-lymphocyte response in ten of 18 subjects, with three demonstrating a complete response (Muderspach et al. 2000). Another trial using a longer E7 peptide with a palmitic acid adjuvant in 12 cervical cancer patients resulted in a 25% T-lymphocyte response rate and a single complete response (Zwaveling et al. 2002). A promising approach involves the use of a non-integrating, non-replicating plasmid encoding multiple HPV 16 and 18 E6 and E7 epitopes, formulated in small biodegradable polymer microparticles with a good profile of safety and tolerability (Amilogene) (Klencke et al. 2002; Sheets et al. 2003). A recent large multicenter randomized placebo controlled trial tested the safety and efficacy of this agent in subjects with histologically confirmed CIN 2 or 3 (Garcia et al. 2004). Of 127 randomized participants for whom cervical conization specimens were available, there was a trend toward higher rates of regression in the active drug group, 43% compared to 27%. More significantly 70% of subjects less than 25 years of age demonstrated histologically-confirmed regression (compared to 23% in the placebo group). A large phase III trial of Amilogene has recently been completed and additional encouraging data from an early phase E7 heat shock protein vaccine have not been published.

The search for therapeutic medical modalities to address cervical intraepithelial neoplasias is particularly important within the larger framework for cervical cancer prevention

(Table 16.2). Chemopreventive and immune therapies open the door for potentially non-surgical, fertility sparing, minimally morbid interventions especially for young women who are burdened by these diseases. These efforts also provide a vehicle for an even more profound understanding of HPV infection, which will serve future generations of women.

### 16.8.3 HPV Prophylactic Vaccines

The development of prophylactic vaccines present the first realistic opportunity for primary prevention. Two prophylactic HPV vaccines have been developed based on the recombinant expression of the L1 major capsid protein, and subsequent self-assembly into virus-like particles (VLPs) that resemble the outer shell of the virus. VLPs are synthetic and contain no DNA and are not live/attenuated viruses. Injection of the HPV VLPs elicits a strong and sustained type-specific response (Future II Study Group 2007a,b). One of the vaccines, Gardasil® (Merck & Co., Inc.), protects against HPV types 6, 11, 16 and 18 (quadrivalent vaccine) and the other, Cervarix (GlaxoSmithKline), protects against types 16 and 18 (bivalent vaccine). The goal of prophylactic vaccination is to reduce the incidence of HPV-related cervical, as well as vulvar, vaginal and anal premalignant and invasive disease, and the diagnostic and therapeutic interventions associated with these disease entities. The quadrivalent product is also expected to protect against genital warts, which potentially should be associated with a reduction in vertical transmission associated with laryngeal papillomatosis.

A series of well-designed industry sponsored trials have demonstrated the prophylactic vaccines for HPV types 16 (Koutsky et al. 2002), 16 and 18 (Harper et al. 2004, 2006; Dubin 2005), and 6, 11, 16 and 18 (Barr 2005, 2006; Villa et al. 2005) to be effective for the prevention of persistent HPV 16 and 18 infections and HPV 16- and 18-related CIN 2/3 (Mao et al. 2006). The populations studied in these trials restricted the age, lifetime number of sex partners, past history of cervical abnormality, and prevalent HPV 16 or 18 infections. In this population however, at more than five years of follow up, clinical studies consistently demonstrate near 100% efficacy in the prevention of persistent type-specific HPV infections and CIN 2/3 among subjects adherent to the study protocol (per protocol analysis group) and who did not have evidence of the specific viral type found in the vaccine formulation prior to prophylactic vaccination. The quadrivalent product also protected against HPV 6-, 11-, 16- and 18-related external genital lesions including genital warts and vulvar and vaginal neoplasia. While for women with normal cytology at baseline and no carcinogenic HPV types within 90 days of study enrollment, the bivalent vaccine reduced the rate of HPV 16/18-associated abnormal cytologic results by 93%. Although the ultimate goal of these products is to prevent malignancy, persistent HPV infection and infection related CIN 2/3 were used as valid and appropriate intermediate clinical endpoints due to ethical and practical considerations which preclude the use of invasive disease as an endpoint.

The Females United to Unilaterally Reduce Endo/Ectocervical Disease-II (FUTURE II) trial focused soley on high-grade cervical disease (i.e., CIN 2 or 3, adenocarcinoma in situ [AIS], cancer) endpoints (Future II Study Group 2007b). After three years of follow-up, 12,167 women (age 15 to 26 years) who completed the vaccination regimen

per protocol and were negative for the respective HPV vaccine type at entry through one month following the third vaccine dose, vaccine efficacy was 98% (95% confidence interval [CI]: 86–100) for preventing HPV 16- or HPV 18-related CIN 2/3 and AIS, with only one case of CIN 3 in the vaccine group compared to 41 cases of CIN plus an additional AIS case in the control group.

By contrast, FUTURE I (Garland et al. 2007) considered both cervical and vulvovaginal disease endpoints. In three years of follow-up of 5,455 subjects (age 16–23 years) who completed the quadrivalent vaccine regimen, did not violate the protocol and who had no virological evidence of infection with the specific HPV vaccine type at study entry through one month following the third vaccine dose, the vaccine prevented 100% (95% CI: 94 to 100) of HPV 6-, 11-, 16-, and 18-related cervical lesions of any grade.

An intent to treat (ITT) analyses was conducted to evaluate the impact of the quadrivalent vaccine with respect to HPV 6-, 11-, 16-, and 18-related cervical in all women randomized in both trials and received at least one dose of vaccine. The goal was to estimate the overall vaccine impact on prevalent disease regardless of baseline HPV status (i.e., prevalent infection at study entry) and sero-status (i.e., prior infection). This included events arising from HPV infections and disease related to the vaccine specific HPV present at time of vaccination as well as those arising from infections that were acquired after vaccination. Impact was measured starting one month post-dose one, with three year follow up.

The majority of CIN and detected in the group that received quadrivalent vaccine occurred as a consequence of HPV infection present at enrollment. In FUTURE II (Future II Study Group 2007b), efficacy for HPV 16- or 18-related CIN 2/3 or AIS was estimated at 44% (95% CI: 26–58%), with 83 cases of high grade disease in the vaccine arm compared to 148 among the placebo arm. For FUTURE I (Garland et al. 2007) the efficacy for HPV 6-, 11-, 16-, or 18-related CIN or AIS was 55% (95% CI: 40–66%), with 71 and 155 events in the vaccine and control arms, respectively. An interim analysis of combined phase II and III quadrivalent vaccine studies (median follow-up of 1.9 years) demonstrated a 12.2% (95% CI: −3.2–25.3%) reduction for CIN 2/3 (compared with placebo) regardless of HPV type (Miller 2007). As would be expected, when subjects entered these studies with evidence of current or past HPV infection, by PCR- or serology-positive for HPV related vaccine types, there was no protection from subsequent disease with prophylactic quadrivalent vaccination (Hildescheim et al. 2007).

The phase II bivalent vaccine trial provides information regarding vaccine efficacy and durability of the immune response. Approximately 776 women (age 15–25 years) who completed the three-dose vaccination regimen were followed for 25–53 months (mean follow-up was 48 months). Vaccine efficacy was 100% (95% CI: 42.4–100%) for preventing HPV 16- or HPV 18-related CIN 2 or 3; this included no cases in the vaccine group and five cases in the placebo group. Additionally there was a single case of persistent HPV 16 or 18 among vaccinated women compared to 23 cases among controls receiving placebo (96% efficacy) (Harper et al. 2006).

An interim analysis of the placebo controlled trial of the bivalent product involving 18,644 young women (aged 15–24) who completed the 3-dose vaccination regimen (per protocol group) and participated in an extended follow-up study, demonstrated efficacy of 93 and 83% for preventing HPV 16- or HPV 18-related CIN 2 or greater, with a total of two cases in the vaccine group and 21 in the placebo group (Paavonen et al. 2007).

The results failed to reach significance for HPV 18 due to the low number of events. The efficacy for preventing persistent (12 months) HPV 16 or 18-related, the obligate precursor of high grade cervical disease and cancer, was estimated to be 80% for HPV 16 and there was a trend toward significance for HPV 18 and the related viral types 33, 45, and 52.

There are limited data on the long-term duration of HPV vaccine-induced immunity, and no practical immune correlates of vaccine or naturally induced immunity that might be useful in estimating this.

Based on these clinical trial data, the greatest cervical cancer prevention benefit of this primary prevention intervention will likely be for young women (median age of 16 years) reporting on average two (and no more than four) lifetime sexual partners at vaccination. In an ideal setting vaccination prior to sexual intercourse would be implemented to achieve optimal effectiveness with regards to the public health endpoint of cervical cancer prevention. However, identifying this population in clinical settings is problematic for many reasons and public health policy strategy should target individuals based on age thresholds at which exposure is likely to occur. One challenge is that about a quarter of girls report being sexually active by age 15, 40% by age 16, and 70% by age 18 (Abma et al. 2004). Additionally, 8% of high school students report initiation of intercourse before age 13, and 10% of sexually active ninth graders reported having had 4 or more lifetime sex partners (CDC 1999). Current population based estimates suggest that women between the age of 20 and 49 years have a median number of six lifetime partners (Nagelkerke et al. 2006).

The risk of exposure to carcinogenic and non-carcinogenic HPV types increases with number of lifetime sex partners (Vaccarella et al. 2006). Given that HPV acquisition is a marker of the onset of sexual activity it is not surprising that among 13–21 year old women, 70% had evidence of HPV infection within five to seven years of onset of sexual intercourse (Moscicki et al. 2001). For college age women, 39% will acquire HPV within 24 months of onset of sexual activity (Winer et al. 2003).

Unlike vaccine performance in younger populations which will likely mirror clinical trial findings, the efficacy and potential cervical cancer prevention benefit of HPV vaccines after the age of 19 years is less compelling at this time. Certainly women older than 19 years of age are who have not commenced sexual activity will derive the full cervical cancer prevention benefit from HPV vaccination. For women 19–26 years of age who have not been exposed to all four HPV types in the vaccine, there will likely be a cancer benefit. However, many currently and/or previously sexually active women in this age group who have been exposed to HPV 16 or 18 and will have less cervical cancer prevention benefit from prophylactic vaccination,. This population is not easily identified given the current lack of a clinically available HPV typing assay. The public health benefit for HPV 6 and 11 related condylomatous vaginal and vulvar disease derived from quadrivalent vaccination, is not in dispute for this population segment.

Based on such information the American Cancer Society guidelines support the vaccination of girls and women up to 18 years of age. They also advise available evidence is insufficient to recommend for or against vaccination of women age 19 through 26 years of age, and no evidence for women over 26 years of age (Saslow et al. 2007). The ACS prophylactic vaccination recommendations are summarized in Table 16.3.

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## 16.9 Conclusion

Cervical cancer is a potentially devastating disease with major emotional and economic implications for women and their families. Cervical cancer is arguably the best understood of any malignancy, and its etiologic agent, although ubiquitous, is well characterized and typically innocuous. Moreover, a cost effective screening intervention is generally available for the detection of pre-invasive disease. At least theoretically, cervical cancer is entirely preventable given the tools available to practitioners today. Efforts should therefore be aimed at bringing the significant numbers of under and unscreened women into the screening pool and providing clinical services that facilitate their accurate diagnosis and adequate treatment prior to the point of developing invasive disease. Such women are in general medically underserved and uninsured, and bringing them into the health care system presents challenges that are at least as formidable as those presented by drug development.

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# Ovarian Cancer Prevention

# 17

S.K. Chambers, L.M. Hess

## 17.1 Epidemiology

Ovarian cancer is the leading cause of gynecologic cancer death worldwide (Harries and Gore 2002). It accounts for about 22,430 cancer diagnoses and 15,280 deaths annually in the US (Jemal et al. 2007). However, the incidence rate of ovarian cancer varies internationally (Fig. 1), with lower incidence in Egypt and Japan and higher rates among women in Iceland, northern Europe and North America. It is likely that these variations are due to a number of inherited and environmental factors which have yet to be understood. The potential contributing factors related to ovarian cancer risk are described in more detail below.

Despite advances in surgical care and in pharmaceutical treatments for ovarian cancer, the mortality rate from this disease remains high. Because of the lack of distinct warning signs that might suggest cancer, most patients (roughly 75%) have advanced disease at the time of diagnosis (Goodman et al. 2003). Since approximately 80% of all cancers occur in the absence of family or genetic risk factors, the need to be alert to new onset, persistent symptoms cannot be understated. Although often called a clinically 'silent' disease, ovarian cancer is frequently associated with symptoms that are consistent with those of menstruation (e.g. pelvic, back or abdominal pain, constipation, indigestion, frequent urination), but are more severe and persist longer than would be expected (Goff et al. 2004). The Gynecologic Cancer Foundation (GCF), Society of Gynecologic Oncologists (SGO) and American Cancer Society (ACS) issued a consensus statement in 2007 (GCF, SGO and ACS 2007), noting the most common symptoms of early ovarian cancer as:

Bloating

- Pelvic or abdominal pain
- Difficulty eating or feeling full quickly
- Urinary symptoms (urgency or frequency)

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Physicians and women should be aware that persistent (e.g. daily) gastrointestinal or urinary symptoms lasting longer than several weeks suggest the possibility of a gynecologic malignancy, although many other disorders could present with these symptoms as well, as the specificity of symptomology is low. Patients suspected to have a gynecologic malignancy should be referred to a gynecologic oncologist for evaluation (SGO 2000). Appropriate care from the time a malignancy is first suspected provides patients with the best outcome possible if they are diagnosed with this disease and reduces unnecessary surgical and medical procedures. Appropriate care, which includes appropriate surgical staging and optimal cytoreduction, must be performed by board-certified physicians trained in these procedures to ensure they

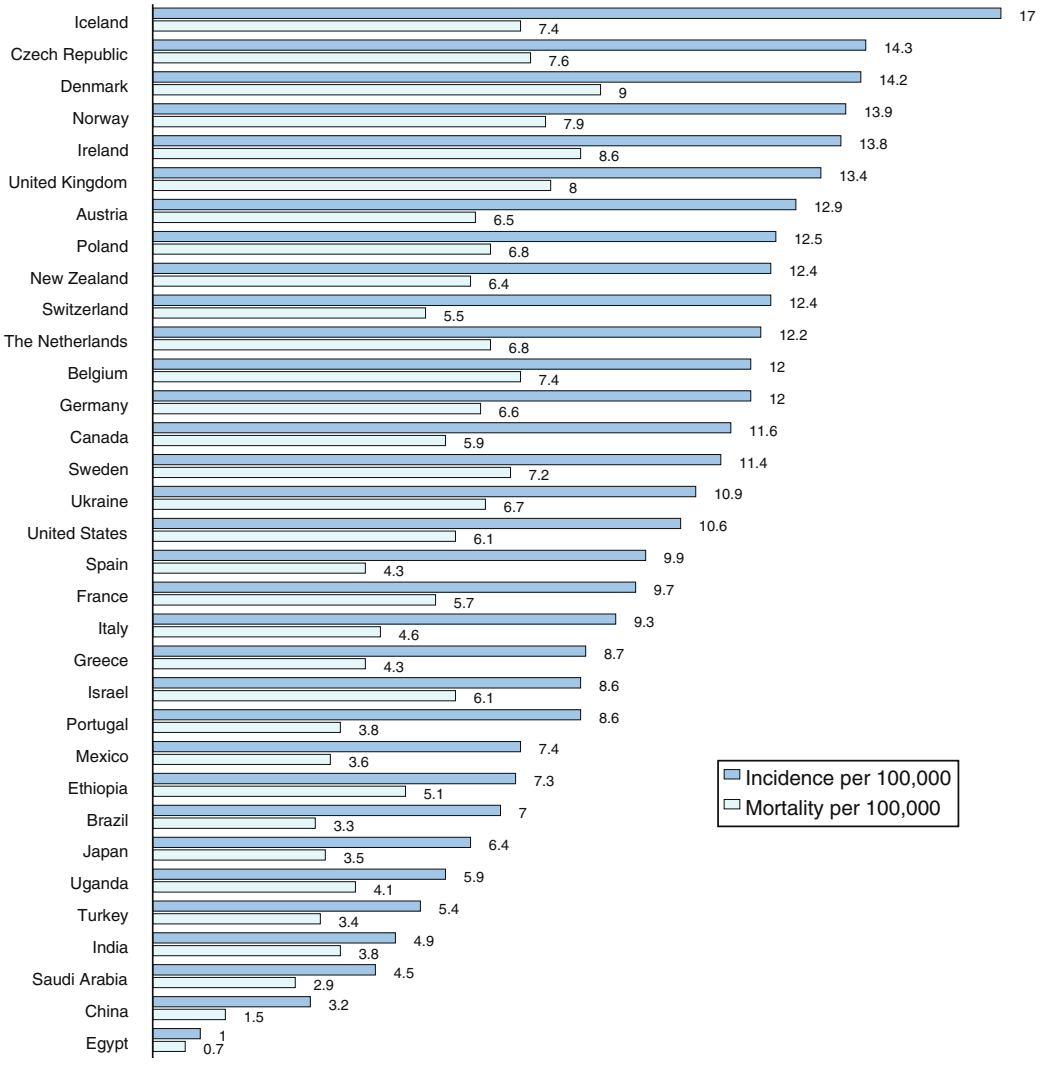


**Fig. 1** Age-adjusted incidence and mortality rates from ovarian cancer, selected countries (IARC 2002)

are done comprehensively and accurately. In the US, only gynecologic oncologists have this certification. The National Institutes of Health (NIH) Consensus Panel issued a statement in 1995, (NIH 1995) which states that all patients suspected to have ovarian cancer be offered preoperative consultation with a gynecologic oncologist. This recommendation is also supported by the American College of Obstetricians and Gynecologists (SGO 2000). Since the vast majority of patients with early stage disease (more than 90%) and many patients with advanced disease (approximately 30%) do not receive appropriate care, patients often experience unnecessary loss in fertility, increased risk of complications, the need for additional surgery, and increased morbidity and mortality (SGO 2000).

While a number of prognostic factors influence ovarian cancer survival (e.g. appropriate surgical care, stage of disease at diagnosis, age at diagnosis), if diagnosed in its early stages, ovarian cancer is curable in a high percentage of patients (Table 17.1) (McGuire et al. 2002). Therefore, advances in the early detection, risk reduction and prevention of gynecologic malignancies have a great potential to reduce the morbidity and mortality associated with this disease.

## 17.2 Histopathology

Ovarian cancer is not a single disease; there are 30 types and subtypes of ovarian malignancies, each with its own histopathologic appearance, biologic behavior and possible etiology (Hildreth et al. 1981). Ovarian malignancies are categorized into three major groups: epithelial; germ cell; and sex cord-stromal tumors. Ovarian cancer that begins on the surface of the ovary (epithelial ovarian carcinoma) is the most common type; more than 85% of ovarian cancers are epithelial cancers, which have been thought to originate in the ovarian surface epithelium (Bai et al. 2000). Malignant germ cell tumors and sex cord-stromal tumors are less common. Carcinosarcoma of the ovary, defined by the presence

**Table 17.1** Five-year survival rates by stage (Rosenthal and Jacobs 1998)

| FIGO stage | Proportion of cases (%) | 5-year survival (%) |
|---|---|---|
| Ia | 19.3 | 92.1 |
| Ib | 2.7 | 84.9 |
| Ic | 8.1 | 82.4 |
| IIa | 2.7 | 69.0 |
| IIb | 4.2 | 56.4 |
| IIc | 3.0 | 51.4 |
| IIIa | 6.9 | 39.3 |
| IIIb | 6.6 | 25.5 |
| IIIc | 18.0 | 17.1 |
| IV | 28.3 | 11.6 |

of malignant epithelial and mesenchymal elements, accounts for fewer than 1% of ovarian malignancies.

More recent work raises the hypothesis of an extraovarian epithelial origin of ovarian cancers (Jarboe et al. 2008). In *BRCA* mutation carriers, serous malignancies previously attributed to the ovary or peritoneum may in fact originate from fimbria or ampulla of the fallopian tube. All seven cancers found in the prophylactic surgical specimens of 122 *BRCA* positive patients were primary fallopian tube cancers (Callahan et al. 2007). These lesions were frequently associated with an intraepithelial component of the tube (TIC or tubal intraepithelial carcinoma) as shown in Fig. 2. Among fallopian tubes containing TICs, there is evidence of a p53 signature consistent with a serous carcinoma precursor that has been detected in the distal fallopian tube, specifically among women with *BRCA* mutations (Lee et al. 2007). Although this work needs further confirmation, there remains the possibility of a molecular lesion in the fallopian tube that is a precursor to serous ovarian, peritoneal or tubal carcinoma that may be useful in future efforts to develop screening tools for this disease. Although the exact location of origin differs, ovarian, peritoneal and tubal cancers act similarly and in many ways are indistinguishable, in terms of their clinical or pathologic presentation, and receive the same treatment regimen. It is generally impossible to make a clear distinction between an ovarian, tubal or peritoneal carcinoma, and they could be considered pelvic serous carcinomas (Barda et al. 2004).

## 17.3 Risk Factors for Epithelial Ovarian Cancer

In general, a woman is considered at high risk of ovarian, fallopian tube or primary peritoneal cancer if she has a *BRCA1* or *BRCA2* mutation or has a family history of ovarian cancer (Holschneider and Berek 2000; King et al. 2003). However, a variety of other reproductive, menstrual, hormonal, dietary and genetic factors contribute to a woman's

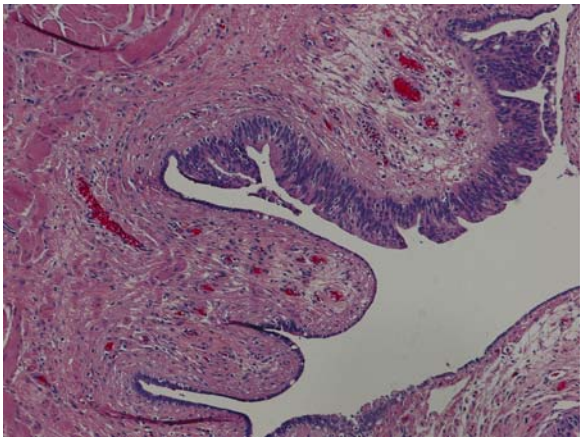

**Fig. 2** Fallopian tube with tubal intraepithelial carcinoma (TIC)

lifetime risk of developing ovarian cancer. In general, protective factors include multiparity, increased time spent breast feeding, oral contraceptive (OC) use, and tubal ligation or hysterectomy (Holschneider and Berek 2000), while risk factors include increasing age, unexplained infertility, endometriosis, nulliparity, family history of ovarian cancer, and certain inherited genetic mutations (e.g. *BRCA 1/2*).

*Age*. Similar to most other cancers, epithelial ovarian cancer is a disease associated with aging (Yancik 1993). Incidence begins to rise in the late teenage years and gradually increases with age (Table 17.2). After age 45, incidence and mortality rise sharply and the highest incidence occurs among women from 80 to 84 years of age (61.8 per 100,000 women) (Edmondson and Monaghan 2001). Ovarian cancer is most frequently (at least 85% of cases) diagnosed among peri- and post-menopausal women (Choi et al. 2007).

*Endogenous and exogenous hormones*. While certain reproductive factors, such as multiparity and breastfeeding, may offer protection against ovarian cancer, others, including early onset of menstruation, nulliparity, and having a first child after age 30, may increase risk. Thus, factors that reduce the total number of ovulations reduce ovarian cancer risk and those that increase the number of ovulations tend to increase risk. In the early 1970s, Fathalla (Fathalla 1971) theorized that the ovarian surface epithelium experienced trauma with uninterrupted, repeated follicular rupture and repair cycles. Fathalla's Incessant Ovulation Theory suggested that the opportunities for genetic alterations introduced by this repetitive trauma were key factors leading to ovarian cancer. This theory provided the biologic rationale for epidemiologic findings that demonstrate a positive association between ovarian cancer risk and lifetime number of ovulations. The risk of ovarian cancer is reduced with early childbirth (e.g. first pregnancy at age 25 or earlier), and is increased with late childbirth (e.g. first pregnancy after age 35) (Negri et al. 1991; Daly and Obrams 1998). Nulliparous women are also at significantly higher risk of ovarian cancer than parous women, regardless of the age of first childbirth (Vachon et al. 2002). In a prospective cohort study of 31,377 Iowa women age 55–69 years, nulliparous women with a family history of first- and second-degree relatives with breast or ovarian cancer were at much higher risk than were their parous counterparts (relative risk, RR = 2.7, 95% confidence interval, CI: 1.1–6.6) (Vachon et al. 2002). There was an increased risk for nulliparous women with family history of breast or ovarian cancer. Ovulation may also be interrupted by the use of OCs and breast feeding, and therefore similar to the number of full term pregnancies, OCs have also demonstrated a protective effect against the development of ovarian cancer (Whittemore et al. 1992).

**Table 17.2** Five-year survival rates by age at diagnosis (Ries et al. 2004, 2005)

| Age at diagnosis | % of all ovarian cancers diagnosed | 5-Year Survival (%) |
|---|---|---|
| < 45 | 12.6 | 72.3 |
| 45–54 | 18.8 | 52.9 |
| 55–64 | 21.5 | 46.4 |
| 65–74 | 20.6 | 35.7 |
| 75+ | 26.5 | 20.3 |

An analysis of pooled interview data on infertility and fertility drug use from eight case-control studies conducted in the US, Denmark, Canada and Australia found that nulligravid women who attempted to become pregnant for more than 5 years, compared with nulligravid women who attempted to become pregnant for less than 1 year, experienced a 2.7-fold increased risk of ovarian cancer (Ness et al. 2002). Significant controversy surrounds the relationships among infertility, fertility drug use, and the risk of ovarian cancer (Sit et al. 2002). These pooled interview data on infertility and fertility drug use found that among nulliparous, subfertile women, neither use of any fertility drug nor use of fertility drug for more than 12 months were associated with ovarian cancer risk (Ness et al. 2002). These data suggest that specific biological causes of infertility (such as endometriosis), and not the use of fertility drugs, may play a role in overall risk for ovarian cancer.

Multiparity and interrupted ovulation appears to decrease the risk of ovarian cancer (Daly and Obrams 1998). The Nurses Cohort Study of 121,700 women found that parity reduced ovarian cancer risk (odds ratio, OR = 0.84; 95% CI: 0.77–0.91 for each pregnancy) (Hankinson et al. 1995). A summary of seven case-control studies found that one full-term pregnancy had a significant reduction on ovarian cancer risk (OR = 0.47) (John et al. 1993). Risk decreased as the number of pregnancies increased; after six full-term pregnancies, the odds ratio was 0.29, with a 95% confidence interval of 0.20–0.42. Risk declined by about 15% for each additional full-term pregnancy (Risch et al. 1994).

The lifetime cumulative duration of ovulation may play a role in the risk of ovarian cancer, with early menarche being associated with increased risk of ovarian cancer in a number of studies conducted in the US and elsewhere (Wu et al. 1988). Late menopause has also been associated with a higher risk of ovarian cancer (Hildreth et al. 1981; Malik 2002), but the data are inconsistent. However, a woman's age at menopause has not been associated with the risk of ovarian cancer in several ovarian cancer case-control studies (Hartge et al. 1988; Schildkraut et al. 2001).

The protective effects of ovulation inhibition, such as by hysterectomy or OC use may decrease the risk of ovarian cancer (Kjaerbye-Thygesen et al. 2006; Merrill 2006). In a case-control study, Rosenblatt and Thomas (Rosenblatt and Thomas 1996) found that the possible protective effect of tubal ligation was greatest in women of parity less than four and that the protective effect was only for clear cell and endometrioid tumors. When Cramer and Xu (Cramer and Xu 1995) combined data from two case-control studies, they found that both tubal ligation and prior hysterectomy were protective.

OCs suppress ovulation by a combined therapy of progestin plus estrogen and have demonstrated the ability to reduce ovarian cancer risk. In a follow-up analysis of the Norwegian Women and Cancer Cohort Study, there were 171 cases of ovarian cancer diagnosed in the 96,355-woman cohort. The risk of ovarian cancer decreased with the use of OCs (p for trend < 0.0001) (Kumle et al. 2003). In Australia, a case-control study examined the effects of OC use (Siskind et al. 2000). After controlling for estimated number of ovulatory cycles, the protective effect of OC use appeared to be multiplicative. There was a 7% decrease in relative risk per year that persisted beyond 15 years of exposure (95% CI: 4.0–9.0%). Even short-term use, up to 1 year, may have an effect (OR = 0.57; 95% CI: 0.40–0.82) (Siskind et al. 2000). Women with pathogenic mutations in the *BRCA1* and *BRCA2* genes may also experience a reduced risk of ovarian cancer with OC use (Narod et al. 1998). Any history of OC use was associated with a 0.5 odds ratio (95% CI: 0.3–0.8).

OC use was protective for both *BRCA1* (OR = 0.5; 95% CI: 0.3–0.9) and *BRCA2* mutation carriers (OR = 0.4; 95% CI: 0.2–1.1) (Narod et al. 1998). A population-based case-control study of 767 women also found that 4–8 years of OC use may reduce the risk of ovarian cancer by approximately 50% in women with a family history of the disease (Walker et al. 2002). However, one must be cautious in continuing OCs in the mid to late reproductive years in the high-risk population because of concerns related to increasing the risk of breast cancer.

Hypotheses regarding the initiation phase of ovarian carcinogenesis suggest that the structural changes during ovulation are not sufficient to confer risk of malignant transformation (Risch 1998). Hormonal influences, including those of estrogen, androgens and progesterone, independent of the number of ovulations, may be a contributing factor to ovarian cancer risk. Estrogens and androgens are theorized to increase ovarian cancer risk, while exposure to progesterone is protective. During menopause, androgen levels rise, and with binding to the surface epithelium, may promote carcinogenesis. Although the exact role of androgens in the ovarian epithelium is unclear, ovarian epithelium contains a high level of androgen receptors (Risch 1998). OCs act as mild anti-androgens, and it is hypothesized that this mechanism could contribute to their protective effect. Importantly, OCs are progesterone-dominant, which further enhances their protective role against ovarian cancer. The excess of progesterone during pregnancy, instead of the cessation of ovulation, may in fact be the protective factor. There is epidemiologic evidence supporting this, as progestin-only oral contraceptives confer a similar protective effect to combination oral contraceptives, yet do not inhibit ovulation (Risch 1998). This suggests that ovulation cessation is not the primary contributing factor to the reduced risk of ovarian cancer associated with long-term use of oral contraceptives.

Other theories of ovarian cancer initiation include the Gonadotropin Theory, which suggests that exposure to gonadotropins (e.g. luteinizing hormone, LH, and follicle-stimulating hormone, FSH) increase ovarian cancer risk. During ovulation, there is an increase in gonadotropins to regulate gametogenesis. The Gonadotropin Theory is in line with the protective effect of pregnancy and OC use and the increased risk of ovarian cancer during menopause, when gonadotropins rise. However, the mechanism by which gonadotropins may increase cancer risk is unknown (Choi et al. 2007). Despite the current lack of knowledge about the mechanism of action of the various hormones implicated in epidemiologic data to date, the hormonal milieu of the ovarian epithelium is currently thought to be an important factor associated with ovarian cancer risk.

There is an association between long-term use (e.g. 10 years or more) of estrogen replacement therapy (ERT) and increased ovarian cancer risk. Estrogens may act as promoters in the carcinogenic process and occasionally their metabolites may act as anti-hormones or have other physiologic effects (Lipsett 1979). However, combination hormone replacement therapy (HRT), which contains both estrogen and progesterone, has not demonstrated consistent findings related to ovarian cancer risk, suggesting that unopposed estrogen formulations are of greater concern, particularly among women who are already at risk of ovarian cancer.

There remains a great deal of controversy regarding the potential risk of ovarian cancer and HRT or ERT. The inconsistency in findings may be in part related to the fact that estrogen formulations in HRT vary in their effects on estrogen-sensitive target tissues, such as

the ovary. Further, the balance between progestin and estrogen is believed to be a key factor. A prospective study of 211,581 healthy postmenopausal women in the US evaluated women who had taken oral HRT or ERT after age 35 to compare ovarian cancer mortality with the effect of HRT (Rodriguez et al. 2001). Risk of ovarian cancer mortality was reported to be higher in HRT users at baseline and slightly higher for previous users than never users. Risk doubled with 10 years or more duration of use; however, only 66 of the 944 women who died of ovarian cancer had used HRT for at least 10 years. Furthermore, most of these 66 women took unopposed estrogen (ERT) during the 1970s and early 1980s, when the use of higher doses of synthetic estrogen was common. Others have also found that long-term, high-dose unopposed ERT may increase the risk of ovarian cancer (Drew 2001). A study of 44,241 postmenopausal women found that those who used ERT, particularly use for 10 years or more, were at significantly increased risk of ovarian cancer (Lacey et al. 2002). Women who used short-term HRT did not experience increased risk. In 2007, data were published from the Million Woman Study (948,576 postmenopausal women in the U.K.), which demonstrated an increased risk of ovarian cancer among women who used HRT, and the rate increased with duration of use (Beral et al. 2007). A study of 655 histologically-verified epithelial ovarian cancer cases and 3,899 randomly selected controls found that risk of ovarian cancer was elevated among ever users as compared with never users of both ERT and HRT (Riman et al. 2002). Ever users of ERT- and HRT-sequentially added progestins, but not HRT-continuously added progestins, may increase risk of ovarian cancer (Riman et al. 2002). The slight declines in ovarian cancer incidence in the US (primarily in white women) has been attributed to the increased use of OCs in the 1980s and the reduced use of HRT in the early 2000s (Espey et al. 2007). Prospective research has suggested the safety of short-term HRT (e.g. 1–2 years) regarding cancer risk among *BRCA1/2* mutation carriers following prophylactic oophorectomy (Rebbeck et al. 2005). The benefit of improved quality of life following oophorectomy with HRT may greatly outweigh any potential risk of short-term use. The decision to use HRT or ERT should be based on the severity of patient symptomology and patient-provider discussion about the possible risks and benefits of HRT or ERT as well as other therapies available for menopausal symptoms (see Quality of Life Section below).

*Genetic risk factors*. Approximately 10% of all ovarian cancers are associated with family history and inherited genetic factors, such as *BRCA1/2* mutations (Claus et al. 1996). Researchers have identified three primary syndromes by which ovarian cancer can be inherited. The syndromes include the Site-Specific Ovarian Cancer Syndrome or the Hereditary Breast/Ovarian Cancer Syndromes (involving mutations in the *BRCA1* or *BRCA2* genes) and the Lynch Syndrome II (involving mutations in DNA mismatch repair genes), which applies to women with female or male relatives who have had non-polyposis-related colorectal (NHPCC) or endometrial cancer (Fig. 3) (Murdoch and McDonnel 2002). Mutations at other loci, such as p53, may explain other inherited ovarian cancer cases (Sellers et al. 1993).

*BRCA1* and *BRCA2* are genes that normally work together to prevent breast and ovarian cancer; however, in some cases a mutated or altered form of *BRCA1* or *BRCA2* is inherited. This mutation interferes with the normal activity of the gene, making individuals more susceptible to both breast and ovarian cancer. Individuals with one of these gene mutations have a higher risk of developing breast and ovarian cancers and may also pass that gene mutation on to his or her children.

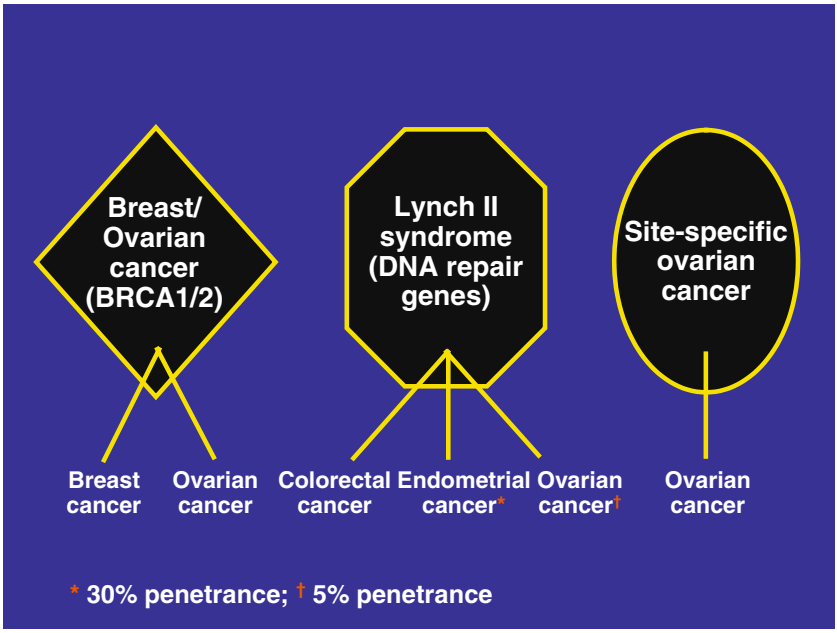


**Fig. 3** Ovarian cancer syndromes

Genetic mutations in the *BRCA* genes (*BRCA1* and *BRCA2*) are inherited risk factors for ovarian cancer that do not fall into either the incessant ovulation or hormonal milieu theories. Women with *BRCA1* mutations have a 47–63% lifetime risk of developing ovarian cancer and a 71% lifetime risk of developing breast cancer; women with *BRCA2* mutations have a 23–27% and 84% lifetime risk of developing ovarian or breast cancers, respectively (Couzin 2003; King et al. 2003; Levy-Lahad and Friedman 2007). These mutations substantially increase personal risk above that of the general female population, which has a lifetime risk of 1.5% for ovarian cancer and 12.7% for breast cancer (Ries et al. 2005). Only one out of every 500–1,000 women from the general population has a *BRCA* mutation (less than 0.2%), while the prevalence is estimated to be one out of every 50 women (at least 2%) of Ashkenazi Jewish ethnicity (Saslow et al. 2007).

The *BRCA* genes seem to work differently in different environments; a number of factors (e.g. reproductive history, hormone therapy, diet, and the presence of other genes which, for example, control the metabolism of hormones) modify the effect of any gene in determining the final outcome. All cancers, including ovarian cancer, are ultimately determined by a combination of genetic and environmental factors.

*Family history*. A family history of ovarian cancer, especially if two or more first- or second-degree relatives have been affected, is associated with an increased risk of ovarian cancer. Ovarian cancers tend to occur at an early age among cancer family members (e.g., before age 50) and tend to be advanced serous epithelial cancers. However, family history of ovarian cancer without a known *BRCA* mutation is also a risk factor. Women with two or more first or second degree relatives who have been diagnosed with ovarian

cancer are at significantly increased risk (risk ratio = 2.12; 95% CI, 1.19–3.78) (Kerber and Slattery 1995).

*Sociodemographic factors*. The incidence rates of ovarian cancer in Hispanic American Indian and African American women were lower than those for White women in the US; however, the lower incidence does not correspond to a lower mortality rate from ovarian cancer among these populations (Ries et al. 2004; Espey et al. 2007). In 2007, slight declines in ovarian cancer were demonstrated among white, non-Hispanic and Asian/Pacific Island women, but similar declines were not seen among African American, American Indian, or Hispanic women (Espey et al. 2007). Poverty, which is associated with ethnicity in the US as well as various parts of the world, may be a risk factor for a number of chronic diseases, including ovarian cancer. This risk may be conferred through an association with reduced access to health care and may contribute to poor outcomes in ovarian cancer due to barriers of cancer diagnosis and treatment.

Founder populations (e.g. those descending from a small group of ancestors) have a significantly greater risk of mutations in *BRCA1* or *BRCA2* as well as many other genetic mutations associated with cancer risk (Koifman and Koifman 2001; Weitzel et al. 2005; Anagnostopoulos et al. 2008; Fackenthal and Olopade 2007). In the US, a greater proportion of women from Ashkenazi Jewish ancestry have *BRCA1* or *BRCA2* mutations (approximately 1 in every 50) (Hartge et al. 1999) than the general population (approximately 1 in every 400) (McClain et al. 2005). The vast majority of *BRCA* mutations are inherited; however, carriers of germline *BRCA1* mutations more frequently will experience somatic mutations in *BRCA2* and vice versa (Welcsh and King 2001). In fact, most sporadic ovarian cancers also demonstrate somatic inactivation of the *BRCA* genes, but lack the point mutations seen in those with *BRCA* hereditary syndromes (Welcsh and King 2001). Although the majority of research among populations with *BRCA* mutations has focused on individuals of Ashkenazi Jewish ethnicity, the founder effect is common among a variety of populations internationally, such as Icelanders (*BRCA2* 999del5 mutation), founder populations from Belgium (*BRCA1* IVS5 +3A > G), African Americans (*BRCA1* 943ins10 and *BRCA1* M1775R), Russians (*BRCA1* 5382insC, *BRCA1* 4153delA), Germans (*BRCA1* 5382insC and *BRCA1* C61G), Hispanics (*BRCA1* 185delAG) and others, and is not limited to the Ashkenazim (*BRCA1* 5382insC, *BRCA1* 185delAG, and *BRCA2* 6174delT mutations) (Neuhausen 2000; Weitzel et al. 2005).

*Diet*. A number of studies have proposed that high dietary fat intake is associated with increased risk of epithelial ovarian cancer; this conclusion, however, remains speculative in part due to the fact that the mechanism by which dietary fat, or even stored fat, increases risk is unknown. The effect of dietary fat may be independent or may act primarily though an influence on hormonal status. Dietary fat consumption appears to impact enteric reabsorption of steroid hormones mediated by the intestinal flora (Mansfield 1993). A meta-analysis of the association between high as compared to low dietary fat intake and risk of ovarian cancer found that high dietary fat intake appeared to represent a significant risk factor in the development of ovarian cancer (Huncharek and Kupelnick 2001). A case-control study in China investigated whether dietary factors had an etiological association with ovarian cancer (Zhang et al. 2002). Controlling for demographic, lifestyle, hormonal status, family history and total energy intake,

ovarian cancer risk decreased with a high consumption of vegetables and fruits and increased with high intake of animal fat and salted vegetables. Risk appeared to increase among women who consumed high fat, fried, cured and smoked food (Zhang et al. 2002). More recently, analyses of the Women's Health Initiative (WHI), a study of 48,835 women who were randomized to either a healthy diet (reduced fat and increased fruit and vegetable intake) or observation, found a statistically significant reduction in the risk of developing ovarian cancer among those randomized to the healthy diet ($p = 0.03$) (Prentice et al. 2007).

*Obesity and Physical Activity*. While obesity is more common in westernized than non-westernized societies, obesity rates vary by race and ethnicity. In the US, over 30% of all adults are obese, according to analyses of the National Health and Nutrition Examination Survey (Flegal et al. 2002). Obesity may be a risk factor for cancer incidence and mortality; this may in part be due to higher levels of circulating estrogens among those with a higher proportion of adipose tissue that contributes to an increased risk of hormone-dependent cancers (Key et al. 2003). However, the relationship is not consistent across all cancers. The risk of mortality due to ovarian cancer has been shown to be higher among those with higher body mass index (a measure of body weight scaled to one's height) (Hoyo et al. 2005; Modesitt and van Nagell 2005; Zhang et al. 2005). Obesity and body fat distribution may increase risk for ovarian cancer, perhaps due to the effect of obesity on estrogen levels. Further, where the body fat is stored and the age at which obesity occurs may also be important in ovarian cancer risk. Women who have a high waist-to-hip ratio and a family history of ovarian cancer experience a 4.83-fold increased risk (95% CI: 1.55–15.1) (Sellers et al. 1993). However, there remains conflicting evidence about these findings, primarily because of the lack of control for potential confounding variables, such as surgical or treatment-related factors.

The role of physical activity and ovarian cancer risk is not understood. Physical activity has demonstrated a protective effect against ovarian cancer in some studies, while it has not demonstrated the same effect in others. A case-control study of women living in Pennsylvania, New Jersey, or Delaware (767 cases and 1,367 controls) found that leisure-time physical activity was associated with a reduction in the incidence of ovarian cancer ($p = 0.01$) (Cottreau et al. 2000). This association remained statistically significant even after controlling for tubal ligation, age, body mass index, family history, and OC use (OR = 0.73; 95% CI: 0.56–0.94). After adjusting for age, parity and other risk factors, a case control study in Massachusetts and Wisconsin (327 cases and 3,129 controls) found no significant reduction in the incidence of ovarian cancer among women who participated in vigorous physical activity (RR = 0.85; 95% CI: 0.39–1.86) (Bertone et al. 2002a).

## 17.4 Early Detection and Prevention of Ovarian Cancer

There are no screening tests for ovarian cancer and there are no known early detection strategies that are feasible to deliver to the general population. Screening for ovarian cancer is difficult because the disease is not highly prevalent in the general population (only 0.075% of the US female population has been diagnosed with ovarian cancer) (Ries

et al. 2004). An effective screening test for ovarian cancer would need have a sensitivity of at least 80% for early-stage, curable disease, a positive predictive value of at least 10% and consequently, a specificity of over 99% (Jacobs 1998). Prior to implementing a population-based ovarian cancer prevention program, the at-risk population needs to be defined. This can only be accomplished currently by questioning the patient about family history. Since only 10% of women with ovarian cancer have a positive family history for ovarian cancer (Schildkraut and Thompson 1988) and genetic testing is not without cost or controversy, additional methods must be developed to better identify women at increased risk of ovarian cancer.

A second major factor regarding the inability of the medical community to identify an effective early detection method is due to the lack of identification of a precursor lesion. Many other epithelial cancers (e.g. cervix, colon/rectum, endometrium) arise from precancerous lesions. For these cancers, progression is thought to occur in a stepwise, reversible pattern from a mildly dysplastic lesion (e.g. colorectal adenoma, hyperplasia without atypia or mild dysplasia) to a highly dysplastic lesion (e.g. advanced colorectal adenoma with high-grade dysplasia, cervical intraepithelial neoplasia III, or complex hyperplasia with atypia) to carcinoma. Screening tests have been designed to identify and remove these precursor lesions (e.g. colonoscopy to remove an adenoma, colposcopy followed by loop electrosurgical excision procedure, or LEEP, to remove cervical intraepithelial neoplasia) before they can become cancerous. Little is known about the origin of ovarian cancers, and no such precancerous or intraepithelial lesion has yet been identified. Although research is moving forward to try to identify a molecular signature that may indicate the future development of cancerous cells (Jarboe et al. 2008), screening at this time can at best be developed to detect existing cancers.

Early detection tests have also not been successful due to the challenge of visualizing or palpating the ovary to detect growths. The ovary is located in the pelvis, closely surrounded by the bladder, uterus, and the rectum. It is not uncommon for an ovarian mass many centimeters in diameter to be completely undetectable to both patient and non-gynecologist physician without the utilization of advanced imaging technologies. Because of the non-specificity of ovarian cancer symptoms, the work up of patients later diagnosed with ovarian cancer is often left to the non-gynecologist. Pap smear, a test to detect cervical dysplasia, cannot detect ovarian cancer unless it has spread through the uterus down to the cervix (a very rare event) where it can be identified during cervical cancer screening.

Most imaging technologies currently available are also not well suited for the early detection of ovarian cancer. Computed tomography (CT) (Anderson et al. 2004) scans and magnetic resonance imaging (MRI) are not sensitive enough for early detection of peritoneal disease (they generally cannot detect lesions less than one centimeter in diameter, especially in the pelvis) and as a result, they tend to have a high rate of false negative results even among women undergoing evaluation for recurrence (van der Burg et al. 1993; De Rosa et al. 1995). CT scans are not sensitive enough to detect a pelvic mass. Although cost prohibitive, positron emission tomography (PET) scans are more sensitive than a CT scan for the evaluation of lesions. The PET scan, like a CT scan, has more value in the setting of evaluation for recurrent disease or for ruling out or confirming the growth of an existing tumor than for general ovarian cancer screening purposes.

Currently, the best imaging technology for patients with indications of ovarian cancer or other suspected pelvic diseases (e.g. fibroid tumor, pelvic inflammatory disease) is transvaginal ultrasound (TVUS), which can generally detect the presence of a mass (Fig. 4). TVUS has a calculated specificity and sensitivity of 98.9% and 81%, respectively (van Nagell et al. 2000). This improvement over other imaging technology is due to the fact that with transvaginal procedures the transducer is placed closer to the ovaries than is possible with abdominal ultrasound procedures (Takahashi et al. 1993). Unfortunately, the positive predictive value is low (22%) even among those at high risk, as it is only able to detect structural abnormalities and is unable to differentiate benign conditions. Furthermore, TVUS is unable to detect peritoneal cancers or cancers that do not affect ovary size or ovarian morphology.

The CA-125 antigen is a reliable marker for initial disease progression or regression in approximately 80% of advanced epithelial ovarian cancer cases (Gladstone 1994; Meyer and Rustin 2000). CA-125 levels are elevated in an estimated 40–60% of patients with early stage disease and are not elevated in patients with mucinous ovarian cancers and some high-grade cancers (Meyer and Rustin 2000; Kozak et al. 2003, 2005). CA-125 is measured each clinical visit to evaluate tumor response to therapy among patients who present with elevated CA-125 at the time of diagnosis (Verheijen et al. 1999). Despite its relative reliability in the established ovarian cancer patient, it is not a useful screening marker due to its lack of specificity. CA-125 levels fluctuate throughout menstrual cycle among premenopausal women (Bon et al. 1999) and are elevated in approximately 20 other benign conditions, some gynecologic and others non-gynecologic (Buamah 2000). CA-125 has only a 21–33% positive detection rate when used as a screening tool in the general population (Jacobs et al. 1993; Hakama et al. 1996). In the high-risk population, research is ongoing within the Gynecologic Oncology Group (GOG-0199, "Prospective

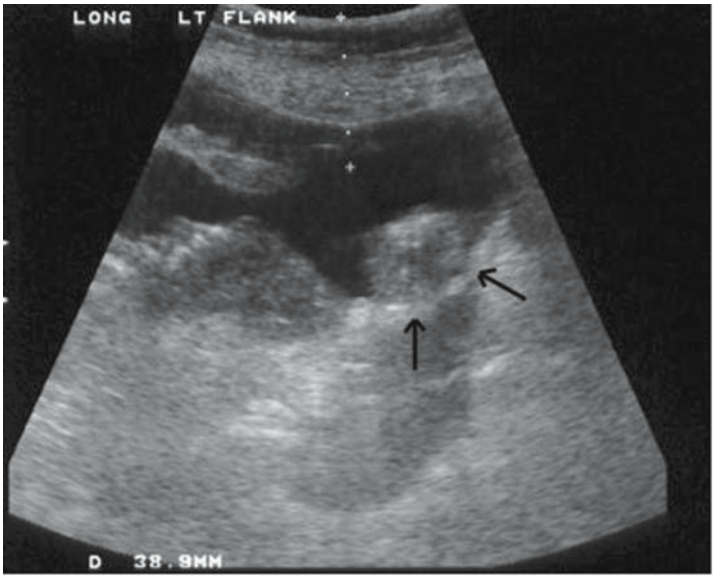


**Fig. 4** Transvaginal ultrasound image of a complex ovarian mass

Study of Risk-Reducing Salpingo-Ooporectomy and Longitudinal CA-125 Screening among Women at Increased Genetic Risk of Ovarian Cancer") to assess the performance of a computerized Risk of Cancer Algorithm (ROCA) in women at increased genetic or hereditary risk of ovarian cancer (Greene et al. 2008). Accrual to this study was completed in 2006, with a total enrollment of 2,605 eligible participants at high risk of ovarian cancer. Of these study participants, 1,030 women were enrolled into the surgical arm of the study, and 1,575 into the screening arm. Estimates of the ROCA screening strategy specificity and positive predictive value will be available in 2011, following completion of the scheduled 5 years of study participant follow up.

Despite their individual limitations, a combined strategy of TVUS, CA-125 and pelvic examination is currently the recommended screening option for women at high risk and who do not elect to undergo risk-reducing surgery. The combination of TVUS and CA-125 in postmenopausal women improves the positive predictive value of screening to 26.8–40%, but still results in many false positives (Jacobs et al. 1993; Olivier et al. 2006). The addition of a pelvic exam (bimanual and rectovaginal examination) increases the likelihood of detecting advanced disease (Olivier et al. 2006). This combined screening strategy has not yet resulted in a reduction in mortality from ovarian cancer (2004). This screening strategy may include the use of imaging technologies when there is substantial risk of ovarian cancer and risk-reducing or exploratory surgery is not a reasonable or preferred option. Unfortunately, only surgery can determine with certainty if a woman has ovarian cancer or if an abnormality detected on imaging studies is benign or malignant.

Proteomics has been explored as potential screening tool for the detection of ovarian cancer. A variety of protein panels are in various stages of development (e.g. Correlogic Systems, Ciphergen, Luminex, and Yale protein panels) (Kozak et al. 2003, 2005; Rapkiewicz et al. 2004; Mor et al. 2005; Cramer 2007; Fredriksson et al. 2008) with preliminary data suggesting they may be at least 80% accurate in the detection of ovarian cancer. The approach to studying specific proteins that may be absent or present in ovarian cancer has primarily involved protein analysis of the serum by antibody microarray (e.g. ELISA) or mass spectrometry (e.g. SELDI-TOF). Although preliminary data are very promising (Mor et al. 2005), additional trials are needed to validate these preliminary results in larger studies before these protein panels can be applied to the clinical care of patients at risk of ovarian cancer. In 2008, the Society of Gynecologic Oncologists (SGO) issued a formal statement cautioning patients and providers from using the FDA-approved serum panels (e.g. OvaSure) for the early detection of ovarian cancer outside of a research trial until validation data becomes available (SGO, 2008).

Although symptoms of ovarian cancer have been identified to occur in some patients later diagnosed with ovarian cancer, as discussed earlier, the identification of symptoms has not improved the early detection or survival of patients with ovarian cancer (Twombly 2007). In most cases, women demonstrating these symptoms do not have ovarian cancer but have symptoms that result from other conditions. Because of the non-specificity of the symptoms of ovarian cancer, women who are later diagnosed with ovarian cancer have often been misdiagnosed several times, told there was nothing wrong with them, or that the symptoms they have been feeling were normal throughout their experience until at some point an imaging or surgical procedure was performed leading to an accurate diagnosis. In addition to the lack of screening and early detection methods currently available, delays in diagnoses occur within the health care system due to misinterpretation of symptoms.

Only half of women later diagnosed with ovarian cancer in the Goff study (Goff et al. 2004) who went to their physicians complaining of symptoms received an accurate diagnosis within 3 months of their first health care visit. Twenty-six percent were diagnosed after 6 months, and 11% were diagnosed after a full year. This suggests that improvements in the healthcare delivery system may be needed to improve the outcomes of women who are diagnosed with ovarian cancer, and that additional work is needed to differentiate between patients with cancer and those with other disorders who have similar symptoms.

At this time, the only known way to significantly reduce the risk of ovarian cancer is prophylactic removal of the adnexae, including both ovaries and the majority of the fallopian tubes (salpingo-oophorectomy). Among women with *BRCA* mutations, prophylactic salpingo-oophorectomy reduces the risk of ovarian cancer by up to 95% and in premenopausal women, reduces the risk of breast cancer by up to 50% (Kauff and Barakat 2007). During prophylactic salpingo-oophorectomy, the ovaries and as much of the fallopian tubes as possible are removed because of the risk of fallopian tube cancer, particularly in women at high risk, because the fallopian tube may function as a point of origin for ovarian cancer. The risk of fallopian tube malignancies is specifically elevated in women with *BRCA* mutations (Callahan et al. 2007). Surgery may also involve a hysterectomy (removal of the uterus). Women at high risk of ovarian cancer (e.g. *BRCA1/2* mutation, Lynch syndrome, family history) are the most likely to be offered the option of a prophylactic salpingo-oophorectomy plus hysterectomy (Rebbeck 2000; Chen et al. 2007). Removal of the uterus reduces the risk of type I endometrial cancer, which is elevated with hormone replacement therapy, which may be needed following oophorectomy (Kauff and Barakat 2007). Patients with *BRCA1/2* or Lynch type II mutations are already at increased risk of type II endometrial cancer (e.g. uterine papillary serous or clear cell cancers) (Lavie et al. 2004; Biron-Shental et al. 2006; Broaddus et al. 2006). The risk is exacerbated in tamoxifen users (Beiner et al. 2007). In addition to the enhanced risk of type I and II endometrial cancers, tamoxifen use also increases the risk of uterine mixed mesodermal tumors (Kedar et al. 1994; Bergman et al. 2000). It is important to note that women who have had hysterectomies for other reasons generally do not have the ovaries removed, so hysterectomy alone cannot prevent the development of ovarian cancer. However, prior hysterectomy may reduce the risk of ovarian cancer by up to 40% (Kjaerbye-Thygesen et al. 2006; Merrill 2006).

Although salpingo-oophorectomy can significantly reduce the risk of ovarian cancer, prophylactic surgery is unable to completely prevent the development of ovarian cancer. Cancer may still develop in the peritoneum at any time following surgical removal of normal-appearing ovaries, so it is not possible to assure that cancer has been prevented. Two to ten percent of patients who undergo oophorectomy without any evidence of disease will still be diagnosed with ovarian or peritoneal cancer at some point following surgery (Tobacman et al. 1982; Piver et al. 1993). Furthermore, 9–17% of patients at high risk have ovarian cancer detected incidentally during the risk-reducing surgical procedure (Morice et al. 1999; Leeper et al. 2002). Therefore, it is very important that prophylactic surgery be performed by a gynecologic oncologist to ensure that appropriate pathologic review of no larger than 2–3 mm sections of the ovary and fallopian tube is thoroughly completed to detect possible microscopic disease (2005). Despite its demonstrated effectiveness in early detection and reducing the risk of future cancers, surgery is not without risk. In addition to the general risks associated with pelvic surgery, removal of the reproductive organs in the pre-menopausal woman is associated with loss of fertility, induced menopause and its symptoms and adverse effects (e.g. bone loss, hormonal changes).

## 17.5 Chemoprevention of Ovarian Cancer

The ability of OCs to prevent ovulation has resulted in their study as an ovarian cancer preventive agent. It is currently estimated that over 50% of ovarian cancers could be prevented by long-term use (more than 5 years) of estrogen and progestin-containing OCs (Stanford 1991; Ness et al. 2000). An additional benefit of OC use may be an increased rate of progestin-induced apoptosis of aberrant epithelial cells as demonstrated in animal models (Rodriguez et al. 1998). However, OC use is contraindicated as women age (e.g. over age 40) due to the increased risk of venous thromboembolism and stroke, particularly among women who smoke. Additionally, OC use may be contraindicated in women at risk of breast cancer due to the slight, but statistically significant increased risk of breast cancer among OC users (Narod et al. 2002). Therefore, it is of interest to assess the use of medications that target similar pathways at a lower risk of adverse effects to explore opportunities for the chemoprevention of ovarian cancer.

Due to the hormone receptors on the ovarian epithelium and based on the hormonal milieu theories of cancer initiation, a number of potential chemotherapeutic agents are under investigation for their role in ovarian cancer prevention. In animal models, progestins have been shown to induce apoptosis, inhibit proliferation and to upregulate TGF-β (Rodriguez et al. 1998). As compared to control and ethinyl estradiol-treated monkeys, a sixfold, statistically significant increase in apoptosis was noted in the ovarian epithelium of monkeys treated with levonorgestrel alone. The degree of apoptosis was not different between ethinyl estradiol-treated monkeys and controls. This demonstrated that exposure to the progestin component of oral contraceptives induced apoptosis in the ovarian epithelium (Rodriguez et al. 1998). The induction of apoptosis may also be the primary mechanism of action for a number of other proposed chemopreventive agents, such as the retinoids (Delia et al. 1993; Ponzoni et al. 1995; Toma et al. 1997), anti-inflammatory drugs (Thompson et al. 1997), and selenium (Thompson et al. 1994; el-Bayoumy et al. 1995).

In 2008, the Gynecologic Oncology Group (GOG) initiated a 60-participant study of the progestin lovonorgestrel (GOG-214, "Phase II Double Blind Randomized Trial Evaluating the Biologic Effect of Levonorgestrel on the Ovarian Epithelium in Women at High Risk for Ovarian Cancer"). This study was designed to evaluate the ovarian and fallopian tube epithelium of women at increased risk of ovarian cancer who have decided to undergo prophylactic salpingo-oophorectomy. The ovarian and fallopian tube epithelia are obtained following 4–6 weeks of treatment with lovonorgestrel or placebo at the time of surgery. Tissue will be evaluated to determine the relative frequency of apoptosis as well as proliferation and expression of TGF-β. This study is expected to be completed in mid-2010.

Vitamin A and its derivatives (retinoids) have been evaluated as potential chemopreventive agents because of their ability to induce differentiation and inhibit cellular proliferation. Naturally-occurring retinoids often require high doses and are associated with significant side effects; therefore, vitamin A analogs have been developed. Fenretinide, *N*-(4-hydroxyphenyl)retinamide or 4-HPR, has been demonstrated to have anti-tumor activity in human ovarian cancer cell lines (Formelli and Cleris 1993). Further studies have demonstrated the anti-proliferative and apoptotic effects of fenretinide (Supino et al. 1996). Clinical trial data from a chemopreventive study of women with early-stage breast

cancer revealed a statistically significant decreased incidence of ovarian cancer in the fenretinide treatment group (De Palo et al. 1995). However, more recent trials (e.g. GOG-190, "An Exploratory Evaluation of Fenretinide as a Chemopreventive Agent for Ovarian Carcinoma") failed to accrue sufficient numbers of high-risk patients in part due to the fear of retinoid toxicity (e.g. night blindness), reducing the appeal of these agents as an acceptable strategy for the chemoprevention of ovarian cancer.

Non-steroidal anti-inflammatory agents (NSAIDs), specifically cyclooxygenase-2 (COX-2) inhibitors due to the high COX-2 positivity in ovarian cancer tumors of patients who do not respond to chemotherapy (Ferrandina et al. 2002), may have a potential role in the chemoprevention of ovarian cancer. The biologic mechanisms may be related to immune enhancement, inhibition of COX and inhibition of apoptosis associated with NSAID treatment (Rodriguez-Burford et al. 2002). An 18-patient cohort study failed to see significant reduction in serum VEGF following 3 months of celecoxib treatment, although the study was not randomized and was likely underpowered to detect statistical significance pre- and post-treatment (Barnes et al. 2005). Research to date has been inconclusive, with some preclinical studies demonstrating a possible protective effect, while case-control and cohort trials of NSAIDs and other analgesics (e.g. acetaminophen or paracetamol) have not consistently seen a protective effect (Cramer et al. 1998; Tavani et al. 2000; Fairfield et al. 2002; Friis et al. 2002). Other anti-inflammatory agents (e.g. omega-3 fatty acid) have been proposed as potential candidates for the chemoprevention of ovarian cancer; however, epidemiologic data have not provided evidence supporting their ability to protect against ovarian cancer (Bertone et al. 2002b; Larsson and Wolk, 2005). Prospective, adequately powered, randomized trials are needed to evaluate these agents for their potential use as chemopreventive agents in ovarian cancer.

Whether inherited or somatic, genetic mutations are necessary for cancer to develop. These mutations cause the turning on of oncogenes and/or turning off of tumor suppressor genes, which then cause a single cell to no longer follow the normal cellular cycle. Instead of normal apoptosis, a cancer cell will continue to split and divide, creating a population of genetically mutated cells. These cells can grow out of control until a tumor is visible. The hormonal, environmental, and inherited factors may simply be promoters or suppressors of a genetic mutation due to some other random or non-random factor or set of factors that have not yet been discovered. Therefore, current ovarian cancer chemoprevention strategies are based on identifying and impacting hormonal and molecular targets. Much work has yet to be done to understand the mechanisms by which these agents may act on the ovarian epithelium and to identify appropriate biomarkers for clinical chemoprevention research trials.

## 17.6 Quality of Life

Premenopausal women at risk of ovarian cancer who elect prophylactic oophorectomy will reduce their risk of ovarian and breast cancer. Surgery should be planned at least a decade prior to the time when ovarian cancer would be expected to develop, yet must be postponed

until the patient is no longer interested in childbearing. Early removal of the ovaries will cause premature menopause due to the sudden loss of estrogen. Women who undergo premature menopause as a result of surgery that results in the removal of both ovaries are usually younger than women who undergo natural menopause; these younger women may experience hot flashes or night sweats that are more bothersome than those that occur at the time of natural menopause. The symptoms of menopause can be mild to severe, and can include a variety of symptoms that can affect quality of life and sexual functioning. For most women, these symptoms will resolve over a period of several years, but in a small minority of women (10–15%), the symptoms may persist for extended periods of time (Tice and Grady 2006). Even if these symptoms are short-lived, they can have a significant impact on patient quality of life. Many women who experience early menopause experience vaginal dryness as a result of the loss of estrogen that had been produced by the ovaries. As estrogen decreases, the walls of the vagina become thinner and more fragile, and vaginal pH level may increase. Vaginal dryness can range from a mild annoyance to having a significant negative impact on sexual functioning, ability to sleep, and overall quality of life.

Many women opt to manage hot flashes with HRT or ERT; however, there is the possibility of increasing risk of cancer with these medications with long-term (Anderson et al. 2004), but not necessarily short-term use of HRT or ERT (Rebbeck et al. 2005). For patients who are already at risk of these cancers, it is prudent to treat mild menopausal symptoms with non-hormonal alternatives. More severe symptoms may require short-term HRT or ERT, depending on the individual patient's risk. Suggested alternatives are summarized in Table 17.3. As with any medication or treatment, there are benefits and risks that should be discussed between patient and provider. These are only summarized below and do not represent all possible benefits or risks. Not all of the suggestions provided have been clinically proven to reduce the symptoms of menopause. Other methods to control the symptoms of menopause include:

- Keeping a diary of when hot flashes and night sweats occur. This will help identify triggers to their occurrence or times when medication loses its effectiveness.
- Keeping the body temperature cool by dressing in layers, using a fan, choosing cold food and drinks, and sleeping in a cool room.
- Exercising regularly. Physically active women report fewer hot flashes than do sedentary women
- Do not smoke.
- Eating a healthy diet, and avoiding dietary triggers to hot flash occurrence (such as spicy/hot foods, caffeine, and alcohol).
- Short-term use of hormone replacement therapy has not been associated with an increased risk of cancer (Rebbeck et al. 2005); in some cases, the benefit of improved quality of life with HRT may outweigh the potential risk.

**Table 17.3** Selected non-hormonal alternatives for the management of the symptoms of menopause

| Medication | Benefits | Risks |
|---|---|---|
| Selective Serotonin Re-uptake Inhibitors (SSRIs) | SSRIs have been shown to substantially decrease hot flash frequency and severity. Typically, short-term use (1 or 2 weeks) is sufficient to determine if an SSRI is going to be beneficial. More prolonged therapy is generally required to obtain maximum symptom control | Restlessness, fatigue, dry mouth, decreased appetite, constipation, difficulty sleeping and nausea. Side effects are greater at higher medication doses |
| Gabapentin | Reported to decrease hot flashes | Side effects may include lightheadedness, mild swelling of the ankles, or difficulty achieving orgasm |
| Clonidine Hydrochloride | Decreases hot flashes. It may be an appropriate second-line treatment for women who decline or can't tolerate SSRI treatment | Side effects may include dry mouth, dizziness, drowsiness, tiredness, lightheadedness, constipation, decreased sexual desire, lethargy, low blood pressure, and difficulty sleeping. Some patients find it difficult to take this medication over a long period of time. Among women participating in clinical trials of clonidine, there have been high dropout rates because of its numerous side effects |
| Belladonna–phenobarbital–ergotamine preparations | Belladonna is reported to decrease hot flashes, and is an FDA-approved treatment for menopausal symptoms | This combination medication has the potential for abuse and addiction because it contains a barbiturate. It may be difficult to take during the day because of its sedative effect. Side effects include dry mouth, dizziness and sleepiness |
| Phytoestrogens | A recent combined analysis of published randomized controlled studies of isoflavones showed no benefit related to the red clover-derived preparations, and provided mixed evidence regarding the effectiveness of the soy-derived preparations, although the benefits from soy are smaller than those seen with HRT.(Nelson et al. 2006) Other experts, reviewing these and additional data, have concluded that the overall evidence does not show benefit from phytoestrogens in the treatment of hot flashes (Tice and Grady 2006). Dietary phytoestrogens, such as black cohosh, may be effective to treat menopausal symptoms, and are not associated with an increased breast cancer risk.(Rebbeck et al. 2007) | Side effects were similar between subjects taking phytoestrogens and those taking placebo in several clinical trials. Although these are considered to be benign, natural, herbal preparations, they do in fact have at least some estrogen-like biologic effects. It is possible that estrogen-like side effects might occur as well |

(continued)

**Table 17.3** (continued)

| Medication | Benefits | Risks |
|---|---|---|
| Vitamin E | Vitamin E was associated with one less hot flash/day in one study. This difference was statistically significant, but is unlikely to be of clinically meaningful | The long-term safety of vitamin E intake is unknown, but doses within USDA guidelines have not shown toxicity |
| Relaxation techniques | Relaxation techniques, including yoga, massage, meditation, leisure bath, and slow, deep, paced respiration, may provide some relief of hot flashes | No known risks |
| Kegel exercises | Kegel exercises are used to strengthen the muscles of the pelvic floor. These exercises, when done regularly, can increase sensation in the pelvic area and strengthen the muscles that support the bladder | No known risks, but may be only minimally effective |
| Moisturizers and Lubricants | Moisturizers can be used for everyday dryness and during foreplay. Replens is a gel that is inserted into the vagina and lasts for 3 days. Gye-Moistrin is another commonly used vaginal moisturizer. To reduce discomfort during sex, couples should use lubricants before vaginal penetration. Water-soluble (not oil-based) lubricants should be used. Examples of these include: KY Jelly, Ortho Personal Lubricant, Astroglide, and ForPlay. Other useful lubricants include Lubrin vaginal suppositories or Lubafax, which can be inserted about five minutes before intercourse. Some women also have found that Vitamin E oil is useful. Vitamin E capsules can be broken open, and the oil applied directly to the vagina. Vitamin E oil should be applied once a day for 1–2 Weeks, then applications should decrease to one or two times per week | |

## 17.7 Conclusion

There is a great deal of work yet to be done to develop effective screening modalities for ovarian cancer. It is unclear where or how ovarian cancer develops (e.g. perhaps the fallopian tube), which is a critical piece of knowledge needed to develop appropriate and effective screening methods for this disease. However, with what is known at this time, the risk of ovarian cancer can be reduced by hormonal manipulation (e.g. oral contraceptive use, multiparity) during the early to middle reproductive years. Maintaining a healthy lifestyle though diet and exercise may also be beneficial to reduce ovarian cancer risk. It is very important that appropriate steps be taken to determine a patient's risk of ovarian cancer. Patients who may be at high risk should be referred to a high-risk clinic and to a genetic counselor for appropriate management. Nutritional and lifestyle counseling are also likely to be beneficial to patients at high risk. The consideration of risk-reducing salpingo-oophorectomy (RRSO) should involve consultation with a gynecologic oncologist and depends on the patient's individual level of risk and completion of all childbearing. For patients at high risk who do undergo RRSO, there is a need to address the quality of life issues that are associated with removal of the ovaries and premature menopause and to be cautious about managing risk of other cancers (e.g. breast, endometrial).

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# Endometrial Cancer Prevention

# 18

F. Garcia, S.K. Chambers

## 18.1 Epidemiology of Endometrial Cancer

There are 200,000 new cases of endometrial cancer diagnosed worldwide each year leading to 50,000 deaths (Mohr et al. 2007). In the U.S., endometrial cancer is the most common malignancy of the lower female genital tract with an incidence of 40,100 per year, nearly four times as common as cervical cancer (Jemal et al. 2008). Despite the relatively high incidence of endometrial cancer, the prognosis for endometrial cancer is better than for most other gynecologic malignancies. Even though endometrial cancer is greater than 1.7 times more common than ovarian cancer, the disease kills less than half as many women. Approximately 7,470 deaths occur due to endometrial cancer each year, compared to 15,520 deaths annually from ovarian cancer (Jemal et al. 2008).

Endometrial cancer can occur in a wide age range, with the median age at diagnosis of 61 years (Plaxe and Saltzstein 1997; Sherman and Devesa 2003). Three quarters of the women diagnosed with endometrial cancer are post-menopausal. In addition to age, race and country of origin also appear to be factors. Eastern Asia and Western Africa have incidence rates nearly ten times lower than North America (IARC 2005). The lowest incidence rates are found in Eastern Asia (2.2 cases per 100,000), Western Africa (2.2 cases per 100,000), and South-Central Asia (2.3 cases per 100,000); whereas the highest rates are found in North America (22 cases per 100,000), Northern Europe (12.2 cases per 100,000), and Western Europe (12.5 cases per 100,000) (IARC 2005).

Eighty-seven percent of all endometrial cancer cases in the U.S. occur among White women (Kosary 2007). However, African-American women with endometrial cancer are often diagnosed later in the course of disease and have a 49% 5-year survival rate as compared to 81% 5-year survival among White women (Kosary 2007).

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Using the California Cancer Registry data, Plaxe and Saltzstein (Plaxe and Saltzstein 1997) found that approximately three quarters of endometrial cancers present as low-grade disease in whites, Hispanics, and Asians. However, only 56% of endometrial cancers in African-American women are low grade at the time of diagnosis. Age-specific rate analysis confirms that white women present with lower grades and at younger ages than do African-American, Hispanic, or Asian women. Survival is also different between racial and ethnic groups. Essentially 90% of endometrial cancer patients who are White could expect 3-year survival. However, only 62% of African-Americans survived 3 years. These data are consistent with that of Liu and colleagues (Liu et al. 1995a), who also found a survival disadvantage among African-American patients. More recent data demonstrates that the survival disparity had not improved much in the past decade and cannot be explained by treatment differences between African-Americans and Whites (Sherman and Devesa 2003). One possibility is that endometrial cancer types differ between African-Americans and Whites.

## 18.2 Types of Endometrial Cancer

There are several types of malignant neoplasms in the uterine corpus, and endometrial cancer comprises a range of entities. The origins of 97% of adenomatous cancers are the endometrium, and the remaining 3% are sarcomas. Endometrial carcinomas are divided into various histologically-defined types. These include estrogen-dependent ednometroid-type endometrial adenocarcinoma (type I) and non-estrogen-dependent (type II) forms of serous and clear cell carcinomas (Boruban et al. 2008).

Most reports refer to type I endometrial cancers, which have the endometrioid histologic subtype, a common entity which is relatively well understood. The endometrioid variant accounts for the majority (more than 80%) of endometrial cancers. This lesion arises in the setting of a relative hyper-estrogenism. The risk factors for type I endometrial cancers are therefore those factors which lead to a tumor microenvironment with more estrogen than progesterone: obesity, polycystic ovarian syndrome, unopposed estrogen therapy, tamoxifen, anovulation, and late menopause. Old age is also a risk factor for this disease as it is for most cancers. Low grade type I endometrial lesions frequently contain estrogen and progesterone receptors.

The precursor premalignant lesion for type I endometrial cancer is complex endometrial hyperplasia with atypia. Atypical endometrial hyperplasia (AEH) is one of a variety of terms used to refer to premalignant endometrial lesions. A Gynecologic Oncology Group (GOG) prospective cohort study (protocol GOG-167) enrolled 306 women with a community diagnosis of either simple or complex AEH. A central pathology review panel reevaluated the community diagnosis of AEH and found that 26% were considered to be less than AEH, 40% were confirmed as AEH, and 29% were diagnosed as carcinoma (Trimble et al. 2006). At subsequent hysterectomy, 42.6% of the community AEH diagnoses were found to have concurrent endometrial carcinoma. Of these cancers, 31% invaded the myometrium (Trimble et al. 2006). The investigators of this study also evaluated the reproducibility

of a diagnosis of AEH. Even among the central pathology review panel within the GOG, diagnoses were unanimous in only 40% of cases. The kappa value for diagnosis of AEH was 0.28, demonstrating very poor reproducibility of the diagnosis (Zaino et al. 2006). This study demonstrates the challenges with clinical decision making among women presenting with AEH.

More recently, the endometrial intraepithelial neoplasia (EIN) classification system has gained interest as a classification system for endometrial lesions (Baak et al. 2005). EIN incorporates both size and lesion architecture (gland area as compared to stroma). EIN is a relative, rather than absolute, standard of cytologic change that is determined by a morphometric D-score, which is described in more detail by Baak and colleagues (Fig. 1) (Baak et al. 2001, 2005; Mutter et al. 2007). Patients presenting with AEH have an approximate 14-fold increased long-term risk of endometrial cancer, and patients with EIN have an approximate 45 times greater risk compared to women without these lesions (Mutter et al. 2008). Beginning in 2007, the GOG is conducting a randomized, controlled phase II evaluation of megestrol in different dose and sequence in the treatment of EIN from a referred cohort of AEH or EIN (protocol GOG-224).

There is an increasing incidence of type II endometrial cancers, such as uterine papillary serous carcinomas, endometrial clear cell carcinomas, or the uterine mixed mesodermal tumors (carcinosarcomas). These aggressive lesions comprise more than 20% of all endometrial cancers (Broaddus et al. 2006). Type II endometrial cancers are not related to obesity or hormone exposure, and usually lack hormone receptors. Type II endometrial cancers are frequently p53 positive. Interestingly, tamoxifen exposure does predispose to development of type II endometrial cancers (Bergman et al. 2000), although it originally was thought to be a risk factor for only type I endometrial cancers. The type II carcinomas are much more aggressive and less predictable. A description of racial disparities by Sherman and Devesa (Sherman and Devesa 2003) documents significantly higher rates of such aggressive cancers as serous adenocarcinoma and clear cell adenocarcinoma (as well as sarcomas) among African-American women. Survival rates are universally low with aggressive forms (50% versus 36% for Whites and African-Americans, with serous or clear cell adenocarcinoma) but survival rates are lower for African-Americans even with the less aggressive endometrioid adenocarcinoma.

There has been significant recent progress made in understanding the precursors of type II endometrial cancer, specifically uterine papillary serous carcinomas, now called endometrial serous carcinoma (ESC). Serous endometrial intraepithelial carcinoma (EIC) was first proposed as a putative precursor lesion of ESC by Sherman and colleagues in 1992 (Sherman et al. 1992) and formalized by Ambros and colleagues in 1995 (Ambros et al. 1995). It was defined as endometrial surface epithelium or glands replaced by frankly malignant cells that are identical to ESC cells without myometrial or stromal invasion

**D- Score** = 0.6229 + 0.0439 x VPS (measure of stroma to glands) – 0.1592 x glands OSD (outer surface density) – 3.9934 x SDSNA (standard deviation of the shortest nuclear axis)

**Fig. 1** Morphometric D-score calculation (Baak et al. 2001)

(Ambros et al. 1995; Sherman et al. 1992; Spiegel 1995). However, the term "EIC" was challenged by subsequent findings that EIC is commonly (e.g., 33–65%) associated with extrauterine serous carcinoma or even carcinomatosis (Carcangiu et al. 1997; Goff et al. 1994; Lee and Belinson 1991; Sherman et al. 1992, 1995; Silva et al. 1990; Slomovitz et al. 2003; Wheeler et al. 2000). Serous EIC does not behave like classic intraepithelial carcinomas, such as breast ductal carcinoma in situ or cervical intraepithelial neoplasia. Therefore, serous EIC is currently being considered as an early form ESC (Zheng and Schwartz 2005), given the fact that serous EIC and ESC share many features of morphology, molecular biology, clinical behavior, and management (Liang et al. 2004; Sherman et al. 1995; Sherman 2000; Tashiro et al. 1997b; Zheng et al. 1998, 2004).

ESC can arise not only in atrophic endometrium, but also can arise in weakly proliferative or proliferative or even hyperplastic endometrium depending on the hormonal status of the individual patient (Zheng et al. 2004). The association of ESC with atrophic endometrium was first described in early 1980s when hormone replacement therapy was not popular (Hendrickson et al. 1982; Liang et al. 2004). In contrast, hormone usage is common in postmenopausal women. Therefore, ESC is not uncommonly associated with the non-neoplastic endometria being proliferative or non-atrophic.

In that serous EIC is now considered an early form of ESC, the pre-cancerous steps between the benign endometrium and this early form of serous carcinoma have been recently investigated. Since 2004, the entity Endometrial Glandular Dysplasia (EmGD), which bridges the benign resting endometrium to serous EIC, has been described (Liang et al. 2004; Zheng et al. 2004, 2007). Morphologically, the degree of nuclear atypia of EmGD falls short of serous EIC and immunophenotypically, the score of p53 and MIB-1 index are distinctly different from EIC. This is a distinct entity and studies of EmGD has also been recently extended to its association with another subtype of type II endometrial cancer, endometrial clear cell carcinoma (Fadare et al. 2006).

The molecular markers of type II endometrial cancers, such as p53, or MIB-1, could serve as diagnostic adjuncts in screening for patients at risk for type II endometrial cancers. If an endometrial biopsy is warranted on clinical grounds then application of immunohistochemistry for these markers may be helpful, in addition to careful morphologic evaluation. There is currently no known role for transvaginal ultrasound screening in the general population for this subtype. Because these lesions are estrogen and progesterone receptor negative, hormonal means of chemoprevention do not apply.

## 18.3 Risk Factors for Endometrial Cancer

The Hispanic population is the most rapidly growing ethnic group in the United States (del Pinal 1996). The incidence and survival curves for Hispanic women with endometrial cancer are similar to their white counterparts. One potential racial difference is that high-grade disease may have a peak incidence slightly earlier in Hispanics compared to Whites and African-Americans (late sixties versus mid-to-late seventies) (Plaxe and Saltzstein 1997). In contrast to cervical cancer, which is relatively prevalent in Hispanics, endometrial cancer

is not different in the Hispanic population than other non-African-American groups. The increasing incidence of obesity in this ethnic group, however, may predict future disparities in type I carcinoma.

The risk of developing endometrial adenocarcinoma is based largely on the fact that it is an estrogen-dependent cancer (type I endometrial cancers). Factors that tend to increase estrogen levels tend to be associated with increased risk and factors that either lower estrogen levels or balance estrogen with progesterone tend to lower risk (Liu et al. 1995a). Factors that may increase risk are obesity, diabetes, polycystic ovary syndrome, feminizing ovarian tumors, nulliparity, menopause after age 52 years, unopposed estrogen replacement, and tamoxifen treatment lasting longer than 2 years. By contrast, factors that reduce relative risk include normal weight, regular ovulation, combination estrogen-progesterone oral contraceptive pills, multiparity, menopause before age 49 years, and progestin therapy. Several of the factors on both sides of the risk profile are related physiologically such as obesity, insulin resistance, and the resultant hyperinsulinemia and chronic anovulation that are all associated with polycystic ovarian syndrome (Salehi et al. 2004).

Obesity is a major risk factor for type I endometrial cancer. An early study demonstrated a clear relationship between obesity and endometrial cancer (Swanson et al. 1994). The study included 403 cases and 297 controls and found that those in the top quartile of body weight had a 2.3-fold increased risk of developing endometrial cancer. Interestingly, fat distribution was found to be an independent risk factor. Increasing upper body obesity (determined by waist-to-hip circumference ratio, WHR) is associated with increasing endometrial cancer risk. These data were supported by a slightly smaller retrospective study which found a positive correlation between body mass index (BMI) in the highest quartile and endometrial cancer (Goodman et al. 1997a). This later study also began to address the influence that diet may have on endometrial cancer. Based on dietary recall data, cases consumed a higher percentage of their calories from fat than did controls. However, the odds ratio was reduced if dietary history was correlated with BMI at the time of interview. This suggests that obesity itself may be more important than dietary fat intake and that a healthy diet is protective because it reduces the risk of obesity.

Women with diabetes mellitus may be at an increased risk of developing endometrial cancer. Data obtained through the Iowa Women's Health Study have been very useful and provide additional insight into endometrial cancer (Anderson et al. 2001). A positive correlation between increasing BMI or WHR and endometrial cancer was found. This study also found positive associations between endometrial cancer and nulliparity or diabetes, and a negative association with use of oral contraceptives (OCs). The association with diabetes is attenuated when BMI is accounted for in the analysis. However, consideration of BMI does not completely remove the added risk of diabetes in the population studied. The mechanism by which diabetes may contribute to risk of endometrial cancer is not understood. Higher circulating insulin levels in type II diabetics may lead to insulin-mediated activation of IGF-1 receptors in the endometrium (Corocleanu 1993; Ordener et al. 1993; Murphy 1994; Thiet et al. 1994; Irwin et al. 2001). However, this hypothesis was not supported by other data that did not find these differences in endometrial cancer cases compared to controls (Troisi et al. 1997; Weiderpass et al. 2003). Diabetes may be an additional risk factor but is probably much less important than the obesity itself that often precedes type II diabetes.

The major impact of obesity on endometrial cancer is thought to be largely mediated by increased circulating estrogens. Several important correlations support the association between estrogen and endometrial cancer. Epidemiologic studies reviewed by Akhmedkhanov and colleagues (Akhmedkhanov et al. 2001) point very clearly at conditions of relative physiologic hyperestrogenism or prolonged estrogen exposure to the endometrium. Conditions that lower the estrogen to progesterone ratio such as the use of OCs or parity are found to be protective. The protective role of progesterone is highlighted by increased endometrial cancer risk following exposure to unopposed estrogen in post-menopausal hormone replacement therapy and the use of tamoxifen, which functions as an estrogen agonist in the endometrium, in the treatment of breast cancer.

Unopposed estrogen as the mechanism in endometrial carcinogenesis is also supported by the observation of a correlation between age-adjusted endometrial cancer incidence data and menopausal status. Endometrioid adenocarcinoma, the most common histologic variant, is relatively rare prior to menopause (Kosary 1994; Plaxe and Saltzstein 1997). The incidence rises sharply after menopause when the ovarian estrogen and progesterone production decrease dramatically and eventually is discontinued. After cessation of ovarian function, estrogens are still produced in the body by peripheral conversion of andostenedione to estrone by the enzyme aromatase. Aromatase is expressed in adipose tissue providing a very plausible link between obesity and endometrial cancer. A positive correlation has been found between obesity and circulating estrone and estradiol (Judd et al. 1976; MacDonald et al. 1978). Without a similar mechanism for post-menopausal production of progesterone, the post-menopausal state is one of relative increase in the ratio of estrogen to progesterone.

In addition to estrogen in the unopposed state, estrogen is a risk factor for women with prolonged exposure even with physiologic progesterone. Prolonged estrogen exposure can come from early menarche, late menopause, nulliparity, or ovarian dysfunction (MacDonald et al. 1978; La Vecchia et al. 1984; Dahlgren et al. 1991; Brinton et al. 1992). Polycystic ovarian syndrome is one form of ovarian dysfunction in which there is increased circulating andostenedione and increased peripheral conversion to estrone. Polycystic ovarian syndrome is also an interesting entity in relation to endometrial cancer because of its characteristic association with obesity, hyperinsulinemia, and anovulation. Chronic anovulation is an important mechanism by which prolonged estrogen exposure occurs without the benefit of progesterone secreted by the corpus luteum. Persistent estrogen stimulation of the endometrium is also seen in ovarian tumors that secrete estrogen (granulosa cell or theca cell tumors). Yet another risk factor associated with increased lifetime estrogen exposure is nulliparity. Although pregnancy is a state with high estrogens, it is characterized by an increase in progesterone production. In addition, pregnancy is thought to be protective due to its association with relatively fewer ovulatory cycles and the related endometrial proliferation. Nulliparity is a reality in a growing percentage of the female population who make up a large portion of the labor force and are electing to delay childbirth. For obvious reasons, prevention of nulliparity is not a realistic primary prevention strategy.

The use of exogenous estrogens has provided even stronger evidence for the unopposed estrogen mechanism. Estrogen therapy for post-menopausal women is associated with a 2.3-fold risk of endometrial cancer (Akhmedkhanov et al. 2001). The longer the duration of use, the higher the risk. However, menopausal hormonal therapy is not associated with increased risk if both estrogen and progesterone are combined (Rossouw et al. 2002).

Although the use of combined estrogen and progesterone does not increase the risk of endometrial cancer, the combination has not been demonstrated to be protective (Pike et al. 1997). A more recent study supports this conclusion in that addition of progesterone to the estrogen replacement regimen simply negated the increased risk of endometrial cancer conferred by estrogen alone (Archer 2001).

The concept of combined estrogen and progesterone therapy raises the issue of OCs in premenopausal women that are thought to be protective. A large meta-analysis suggests that the protective effect is very modest in that it increases the probability of remaining free of endometrial cancer until age 74 from 97.6 to 98.6% (Schlesselman 1997). A subsequent review of the data (Deligeoroglou et al. 2003) confirmed this modest degree of protection. Furthermore, the protective benefit was not realized until 5 years of OC use were maintained. Interestingly, the protective benefit appears to persist even 20 years after discontinuation of OC use.

It is common practice for patients who present with precancerous lesions (AEH or EIN) to empirically receive progestational therapy. However, there is currently no standard dose or treatment regimen recommended for the care of these women. Furthermore, due to the high risk of concurrent carcinoma (Trimble et al. 2006), most women will undergo hysterectomy. There is a need for both fertility-sparing therapies and improved diagnostic criteria for women with AEH or EIN, as more than half will not have cancer at hysterectomy despite the significantly increased long-term risk of carcinoma. In 2008, the GOG began enrollment to protocol GOG-224, a three-arm randomized trial of women with AEH or EIN. Patients are randomized to receive continuous megestrol 40 mg twice a day (BID) for 12 weeks, cyclic megestrol 80 mg BID for 12 weeks (2 weeks on, 2 weeks off), or no treatment (e.g., immediate hysterectomy). Although the primary goal of this study is to determine the optimal treatment dose and schedule, this study will also identify the rates of regression, progression and carcinoma against which future chemopreventive agents can be tested.

A very important exogenous estrogen source which predisposes to endometrial cancer is tamoxifen. The use of tamoxifen has been shown to be effective in reducing tumor recurrence and prolonging survival for women when used as adjuvant therapy after surgical treatment for ER/PR-positive stage I and II breast cancer (Fisher et al. 1998). In analysis of data demonstrating the effectiveness of tamoxifen treatment for breast cancer, it was found that the incidence of endometrial cancer was 2.53 times greater in the treatment group compared with the placebo group. In this study, all of the participants were part of a study design that included appropriate follow up. Close patient monitoring can partially explain why the endometrial cancers that did occur were almost entirely FIGO stage I or EIN (Table 18.1). Based on these observations, patients receiving tamoxifen without a prior hysterectomy require careful and regular gynecologic follow up and aggressive evaluation of abnormal uterine bleeding. Some have advocated a baseline transvaginal ultrasound to rule out endometrial pathology. Those with a normal endometrium at baseline are less likely to subsequently develop endometrial carcinoma while taking tamoxifen (Goldstein 2002).

Increasingly, dietary factors are being recognized for their important role in cancer prevention. Dietary influences related to endometrial cancer have thus far focused on phytoestrogens, total calorie intake, dietary fat intake, glycemic index, food sources, and micronutrients. Data suggest that positive risk factors for endometrial cancer include a high

**Table 18.1** TNM and FIGO staging of endometrial cancer (Greene 2002)

| TNM category | FIGO stage | |
|---|---|---|
| TX | | Primary tumor cannot be assessed |
| T0 | | No evidence of primary tumor |
| Tis | 0 | Carcinoma *in situ* |
| T1 | I | Tumor confined to corpus uteri |
| T1a | IA | Tumor limited to endometrium |
| T1c | IB | Tumor invades less than one-half of the myometrium |
| T1c | IC | Tumor invades one-half or more of the myometrium |
| T2 | II | Tumor invades cervix but does not extend beyond uterus |
| T2a | IIA | Tumor limited to the glandular epithelium of the endocervix. There is no evidence of connective tissue stromal invasion |
| T2b | IIB | Invasion of the stromal connective tissue of the cervix |
| T3 | III | Local and/or regional spread as defined below |
| T3a | IIIA | Tumor involves serosa and/or adnexa (direct extension or metastatis) and/or cancer cells in ascites or peritoneal washings |
| T3b | IIIB | Vaginal involvement (direct extension or metastasis) |
| NX | | Regional lymph nodes cannot be assessed |
| N0 | | No regional lymph node metastasis |
| N1 | IIIC | Regional lymph node metastasis to pelvic and/or para-aortic nodes |
| T4 | IVA | Tumor involves bladder mucosa and/or bowel mucosa (bullous edema is not sufficient to classify a tumor as T4) |
| MX | | Distant metastasis cannot be assessed |
| M0 | | No distant metastasis |
| M1 | IVB | Distant metastasis (includes metastasis to abdominal lymph nodes other than para-aortic, and/or inguinal lymph nodes; excludes metastasis to vagina, pelvic serosa or adnexa) |

D-score = 0.6229 + 0.0439 × VPS (measure of stroma to glands) − 0.1592 × glands OSD (outer surface density) − 3.9934 × SDSNA (standard deviation of the shortest nuclear axis)

glycemic index (Folsom et al. 2003) and dietary fat consumption (Littman et al. 2001). Some dietary influences that may reduce risk include fatty fish consumption (Terry et al. 2002a), nuts and seeds (Petridou et al. 2002), beta-carotene, vitamin C (Negri et al. 1996), and soy (Goodman et al. 1997b; Lian et al. 2001; Horn-Ross et al. 2003). Phytoestrogens are natural products present in soy beans and soy food products. There are many types of phytoestrogens but the most important ones appear to be genestein and diadzen. These compounds bind to estrogen receptors and have partial agonist or partial antagonist activities. A more specific look at phytoestrogens was provided by Horn-Ross and colleagues (Horn-Ross et al. 2003). In this study, phytoestrogen isoflavones (including genistein and

diadzen) and lignans were associated with a reduced risk of endometrial cancer. Importantly, the intake required to reach this protective effect was not greater than that found in the typical American diet.

Smoking has been shown to lower the incidence of endometrial cancer. In a review of the literature (Terry et al. 2002b), it was found that smokers have a reduced risk of endometrial cancer. This may be explained by an anti-estrogen effect, an idea supported by the observation of a higher incidence of osteoporosis in smokers (Baron 1984; Jensen et al. 1985; Jensen and Christiansen, 1988; Baron et al. 1990). Other possibilities include the association with earlier age of menopause associated with chronic tobacco use, as well as a negative association between smoking and obesity. Given the injurious nature of tobacco use and its role as major cancer risk factor in other organ sites, the protective effect of smoking is unlikely to have any application to primary endometrial cancer prevention.

In addition to the risk factors discussed above, the characteristics of the endometrium itself determine a level of risk for developing endometrial cancer. A continuum of progression from normal to carcinoma exists in the endometrium in an analogous fashion to that described for carcinoma of the colon (Vogelstein et al. 1988) or uterine cervix (O'Shaughnessy et al. 2002). The healthy, cycling, pre-menopausal endometrium will vary between the physiologic states of the menstrual cycle. A very early change along the progression toward cancer is simple hyperplasia of the endometrium. In simple hyperplasia the endometrium is stimulated by estrogen to proliferate but fails to shed completely leaving behind tissue upon which the next level of proliferation builds. Over time, this can lead to a hyperplastic state and abnormal uterine bleeding. Histologic evaluation at this early condition is characterized by normal appearing glands and stroma with increased endothelial cellularity but without cytologic atypia. Hyperplasia with abnormal, crowded glands is termed complex hyperplasia. As with simple hyperplasia, the increased cellularity can exist with or without cytologic atypia in complex hyperplasia. In both simple and complex hyperplasias, the presence of nuclear atypia is a strong risk factor for progression to endometrial adenocarcinoma. The vast majority of hyperplastic states occur in postmenopausal women who may not shed atypical cells in the course of normal menses. In addition, hyperplasia in older women is associated with increased risk of progression (Zaino et al. 1996). The details underlying the specific changes that occur along the continuum are still poorly understood. Future work in this area is likely to provide objective prognostic criteria for women who present with this continuum of disease.

## 18.4 Genetic Factors in Endometrial Cancer

Type I endometrial adenocarcinoma is an estrogen-driven cancer. Several of the risk factors are related to underlying mechanisms associated with hyperestrogenic states within the body and possibly locally within the endometrium as well. Although many risk factors for endometrial cancer are associated with hyperestrogenic states, there must also be underlying genetic predisposition or associated genetic variations. Only a few genes have been implicated in the development of endometrial cancer. Some of these

include receptors for estrogen, progesterone, and androgens. Other genes that may be associated include the oncogenes K-ras and human epidermal growth factor receptor 2 (HER-2)/neu, tumor suppressor genes p53, p21, p16, and PTEN, as well as DNA repair genes hMLH2, hMSH2, and hMSH6. Recently, genes that affect estrogen metabolism have also been implicated.

The ras proto-oncogene functions in the intracellular signaling cascade from some receptor tyrosine kinases. Constitutive ras activity leads to unregulated cell growth and duplication via the mitogen-activated protein kinase cascade. Analysis of 58 endometrial carcinomas and 22 endometrial hyperplasias identified K-ras mutations in 18.9% of carcinomas (Lagarda et al. 2001). All mutations were found in carcinomas; no K-ras mutations were found in hyperplastic endometrium negative for carcinoma. Several earlier studies have suggested K-ras mutations occur early in the course of endometrial adenocarcinoma because somatic mutations can be found in samples of hyperplastic endometrium (Enomoto et al. 1990; Sasaki et al. 1993; Duggan et al. 1994; Tsuda et al. 1995; Ito et al. 1996). However, a complete survey of all endometrial tissue is very difficult and some hyperplasias in earlier studies may have contained small foci of carcinoma.

The oncogene HER-2/neu has been implicated in type II endometrial cancers. Overexpression of HER-2/neu has been demonstrated in 10–30% of all endometrial cancers, but nearly 80% of serous papillary forms (Oehler et al. 2003). HER-2 is one member of a family of tyrosine kinases in which HER-1 is the bona fide epidermal growth factor receptor. No ligand has yet been identified for HER-2. Overexpression of HER-2/neu is associated with increased growth via activation of the pI3K/AKT pathway (Di Fiore et al. 1987; Liu et al. 1995b; Karunagaran et al. 1996; Waterman et al. 1999; Muthuswamy et al. 2001; Menard et al. 2003). Positive HER-2/neu overexpression is associated with a poor prognosis.

p53, an essential protein used by cells to signal DNA damage, is the most studied and most often demonstrated tumor suppressor gene to be altered in a variety of cancers. It can function to delay cell-cycle progression until the damage is repaired or it can induce apoptosis (Vousden and Lu 2002). Mutations in the p53 gene (TP53) are found in approximately 20% of endometrioid carcinomas (Lax et al. 2000) and up to 90% of serous papillary forms (Inoue 2001). p53 mutations are readily found in endometrial carcinoma, but rarely in endometrial hyperplasia suggesting that the role of p53 in pathogenesis occurs rather late in the carcinogenesis process (Salvesen and Akslen 2002). p21 is a downstream target of p53 activity (el-Deiry et al. 1993) and functions through interaction with cyclin-dependent kinases. Decreased p21 has been found in many tumor types including endometrial carcinoma (Palazzo et al. 1997). The $p16^{INK4}$ protein also interacts with the cyclin-dependent kinases. p16 binds to CDK4 and thereby inhibits progression through the cell cycle by inhibiting the CDK4-cyclin D complex (Kamb et al. 1994). Like p21, decreased p16 expression has been demonstrated in endometrial carcinoma (Hatta et al. 1995; Shiozawa et al. 1997; Milde-Langosch et al. 1999; Nakashima et al. 1999). Tumor suppressor genes, such as p53, p21 and p16, are found to be altered in many tumor types and are a necessary step along the path to cancer. By their very nature and integral role in all cell types, they are fundamental to cancer progression but are not tissue specific. Their broad expression makes them excellent targets for therapy aimed at mutant forms; however, they provide little insight into mechanisms of specific cancer varieties. More can be gained from

knowledge of genetic alterations with more limited expression. PTEN (phosphatase and tensin homolog deleted on chromosome ten) is one such tumor suppressor involved in endometrial carcinoma.

PTEN is a tumor suppressor with tyrosine phosphatase activity and sequence homology to tensin, a matrix protein (Li et al. 1997; Steck et al. 1997). Growth factor receptors signal through tyrosine kinase cascades and often involve phosphatidylinositol-3-kinase activity and the generation of the intracellular signaling molecule phosphatidylinositol-(3,4,5)-triphosphate ($PIP_3$) (Risinger et al. 1997; Tashiro et al. 1997a; Latta and Chapman 2002). PTEN removes the 3-phosphate from $PIP_3$ and thus negatively regulates AKT. Negative regulation of the survival-associated Ser/Thr kinase AKT prevents abnormal cell growth. Another key point regarding PTEN is its differential expression in the endometrium during the menstrual cycle (Mutter et al. 2000a,b, 2001). It is highly expressed during the proliferative phase and has been proposed to function in preventing hyperstimulation by estrogen. Estrogen promotes $PIP_3$ production and may induce PTEN expression concurrently as a balance. Loss of PTEN function could then lead to increased $PIP_3$ signaling for survival and growth. Endometrial carcinomas show loss of heterozygosity for PTEN in approximately 40% of cases, and somatic mutations of PTEN have been found in up to 60% of endometrial carcinoma in some studies (Peiffer et al. 1995; Kong et al. 1997; Risinger et al. 1997; Tashiro et al. 1997a). PTEN mutations are most common in type I, estrogen-dependent, endometrial carcinomas but can also be identified in endometrial hyperplasias. This is consistent with an early role for PTEN in the pathogenesis of endometrial cancer (Mutter et al. 2000a,b).

All cancers have an element of DNA damage. This means that alterations in the activity of DNA repair systems must be considered. One well studied DNA repair system comes from investigations into the hereditary nonpolyposis colorectal cancer syndrome (HNPCC) (Dunlop et al. 1997). This is an autosomal dominant cancer syndrome in which many tissues are affected, including the endometrium, ovary and the colon. The genes identified in HNPCC function in the mismatch repair system. The most frequently involved genes include hMLH2, hMSH2, and hMSH6. Although females with HNPCC are at risk for developing cancers in many organs, endometrial carcinoma is the most frequently identified gynecologic cancer in these patients (Watson et al. 1994). Like the tumor suppressor genes, the mismatch repair system is of obvious importance in carcinogenesis; however, they also shed little light on mechanisms of specific cancer types.

More recent studies regarding genetic loci involved in endometrial cancer have begun to look more closely at the role of estrogen metabolism and the activity of estrogen metabolites. Estrogen is synthesized mainly in the ovary but estrogen compounds are also produced by peripheral conversion of precursors via aromatase found in adipose tissue. Subsequently, several metabolites of estrogen are created by the activity of numerous members of the CYP family of enzymes. CYP enzymes are predominantly expressed and function in the liver where they are involved in the metabolism of a wide array of compounds. However, some CYP enzymes are also expressed outside the liver where they can create a local environment of a given metabolite. Relevant for estrogen metabolism, CYP17A1, CYP1A1, CYP11A1, CYP1B1, and CYP3A7 have all been implicated in endometrial cancer (Vadlamuri et al. 1998; Haiman et al. 2001; McKean-Cowdin et al. 2001; Berstein et al. 2002; Lee et al. 2003; Sarkar et al. 2003; Sasaki et al. 2003). Interestingly, the

pregnane X receptor, a steroid receptor, has been proposed to regulate CYP3A expression in response to estrogen stimulation (Masuyama et al. 2003). Estrogen-dependent expression of CYP3A7 is consistent with the demonstrated variation of CYP expression during the menstrual cycle (Sarkar et al. 2003). A local environment of estrogen metabolites may provide a mechanism for estrogen-stimulated progression toward endometrial carcinoma.

## 18.5 Screening and Early Detection of Endometrial Cancer

Although endometrial cancer is the most common malignancy in the female genital tract, it accounts for a relatively low percentage of cancer deaths. The main reason is that most patients present with locally confined disease (i.e., FIGO stages I or II). Most patients present with complaints of post-menopausal bleeding or abnormal uterine bleeding (AUB) if still pre-menopausal. When presented with AUB, the appropriate work-up includes a detailed history and physical examination, as well a transvaginal sonography and assessment of the endometrium by blind endometrial aspiration biopsy, or preferably by hysteroscopy followed by dilation and curettage.

In the near future, evaluation for AUB may include genetic profiling. It was found that genetic expression profiling with cluster analysis accurately places samples into separate clusters that contain either normal or cancer profiles (Smid-Koopman et al. 2004). This result argues that expression profiles are useful. However, this small pilot study of only 12 endometrial cancers and six controls failed to find clusters that correlated with differing grade or stage. Compared to other cancer types, the molecular genetics of endometrial cancer is still relatively under-studied. There are several groups using microarray technology to characterize gene expression profiles in endometrial cancer (Mutter et al. 2000, 2001; Smid-Koopman et al. 2004). It is thought that such profiling could be used prognostically or therapeutically or to detect additional targets for therapy, to characterize individual tumors, or to direct more specific treatments.

Until the cancer genetics field catches up with current tests, evaluation of AUB will continue to rely on histopathologic evaluation. An argument has been for management of this very large population of women by a gynecologic oncologist was recently put forth (Roland et al. 2004). Early screening and diagnosis, however, can easily be accomplished by general gynecologist, and management of these patients by a generalist continues to be the standard of care. The role of transvaginal ultrasound is clear because determination of the endometrial thickness has good negative predictive value among post menopausal women. Endometrial stripes (thickness on ultrasound) less than 5 mm essentially rule out endometrial cancer among post-menopausal women. Stripes greater than five millimeters require additional follow up because the thickened stripe may be due to a variety of benign endometrial conditions, including polyps or hyperplasias of different grades, or cancer.

A second or concurrent step to ultrasound is endometrial sampling. Endometrial sampling may be performed with or without concurrent hysteroscopy. However, although hysteroscopy may be useful to confirm endometrial atrophy in cases of insufficient tissue or endometrial sampling, the utilization of this more invasive procedure does not appear to provide additional information that leads to alteration in the pre-test expected plan of

treatment (Bain et al. 2002). Biopsy results place the endometrium into a category along the continuum between normal cycling endometrium, hyperplasia, hyperplasia with cytologic atypia, or endometrial cancer. Treatment depends on the biopsy results. Simple or complex hyperplasia in women wishing to preserve fertility can be treated with oral progestational agent therapy (Eichner and Abellera 1971; Bokhman et al. 1985; Wentz 1985; Hunter et al. 1994; Kim et al. 1997; Randall and Kurman 1997; Perez-Medina et al. 1999). When fertility preservation is not a concern or among menopausal patients, the spectrum of management may include hysterectomy in women with additional gynecologic pathology (i.e., fibroid uterus, pelvic relaxation, recurrent high-grade cervical intraepithelial neoplasia). An alternative to hysterectomy is dilation and curettage. Dilation and curettage removes only a non-random proportion of the total endometrium and may require repeated future procedures. Endometrial ablation is not recommended in patients with hyperplasia and only should be considered if carcinoma is clearly ruled out due in part to the theoretical concern of burying current or future potential neoplastic lesions. Hysterectomy is required for patients found to have hyperplasia with atypia due to the increased risk for co-existing cancer and progression to cancer. When hysterectomies are performed the uterus should be bi-valved and inspected for myometrial invasion and a frozen section of the uterus should be performed. Tumors found to have invaded more than one half the depth of the myometrium warrant lymph node dissection (Peters et al. 1983; Goff and Rice 1990; Bloss et al. 1991; Kilgore et al. 1995; Mohan et al. 1998).

The prevention of any cancer can occur at the primary level by reducing risk factors. In endometrial cancer the risk factors are essentially all impacted by reducing the lifetime exposure to estrogens. These estrogens can come from endogenous production or from exogenous sources. Unfortunately, even in present day, one exogenous source still seen is in the form of post-menopausal unopposed estrogen therapy. To minimize the risks associated with these factors, it is important that estrogen therapy always occur in combination with progesterone in women with intact uteri. Likewise, premenopausal OCs are safest when in combination form. A non-contraceptive benefit of OC use is the reduced incidence of endometrial and ovarian cancer (Fraser and Kovacs 2003). Of course, other non-contraceptive benefits include reduced menstrual blood loss, decreased dysmenorrhea, and reduced premenstrual syndrome. Reduction of tamoxifen-associated changes in the endometrium and the myometrium can result from subsequent treatment with aromatase inhibitors, or replacement of adjuvant tamoxifen with aromatase inhibitors in post-menopausal women with breast cancer (Morales et al. 2006).

Prevention of endometrial cancer through reduction of endogenous estrogen levels is aimed at reducing obesity. Many risk factors and modifying factors have been mentioned in this chapter, the majority of which are directly impacted by obesity. For example, early menarche, polycystic ovary syndrome, and diabetes are all associated with obesity. High-fat, low-fiber diets are associated with endometrial cancer independently (Littman et al. 2001) even though their relative impact is small when the BMI of the patient is factored into the risk analysis. A similar argument is made for diabetes as a risk factor. The most powerful prevention is likely to come from promotion of balanced, low fat, high fiber diets, exercise, and appropriate surveillance for disease in women with abnormal uterine bleeding. This approach aims to prevent the main risk factor of obesity and diagnose cancers early in their course so as to allow for successful treatment and recovery. Such health promotion needs to start early in life and be reinforced at home, school, as well as in the physician's office.

## 18.6 Conclusion

Endometrial cancer can be a devastating disease with major emotional and economic implications for women and their families. The search for therapeutic medical modalities to address the precursors of these cancers, including premalignant endometrial neoplasias and endometrial glandular dysplasias, is particularly important. Chemopreventive therapies open the door for potentially non-surgical, fertility sparing, minimally morbid interventions especially for young women who are increasingly burdened by these diseases.

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# Cancer Survivorship

# 19

**R. Krouse, N.M. Aziz**

Survival from cancer has improved quite dramatically over the past three decades as a result of advances in early detection, adjuvant and other aggressive therapeutic strategies, and the widespread use of combined modality therapy (surgery, chemotherapy, and radiotherapy). Cancers such as testicular, childhood leukemia, and Hodgkin's lymphoma are now considered amenable to cure, patients with common cancers such as breast or colorectal can look forward to a vastly improved disease free and overall survival, and patients with potentially incurable disease can look forward to living for extended periods of time as a result of better disease control (Meadows 1980; Ganz 1998a; Schwartz 1999; Aziz 2002b; Aziz and Rowland 2003). However, the therapeutic modalities mentioned are associated with a spectrum of late complications ranging from minor and treatable to serious or, occasionally, potentially lethal. One-fourth of late deaths occurring among survivors during the extended survivorship period, when the chances of primary disease recurrence are negligible, can be attributed to a treatment-related effect such as a second cancer or cardiac dysfunction. Most frequently observed sequelae include endocrine complications, growth hormone deficiency, primary hypothyroidism, and primary ovarian failure (Sklar 1999). Also included within the rubric of late effects are second malignant neoplasms arising as a result of genetic predisposition (e.g., familial cancer syndromes) or the mutagenic effects of therapy. These factors may act independently or synergistically. Synergistic effects of mutagenic agents such as cigarette smoke or toxins such as alcohol are largely unknown.

There is today a greater recognition of symptoms that persist following completion of treatment and those that arise years after primary therapy. Both acute organ toxicities, such as radiation pneumonitis, and chronic toxicities, such as congestive cardiac failure, neurocognitive deficits, infertility, and second malignancies, are being described as the price of cure or prolonged survival.

Generally, long-term cancer survivors are defined as those individuals who are five or more years beyond the diagnosis of their primary disease. Long-term effects refer to any side effects or complications of treatment for which a cancer patient must compensate;

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also known as persistent effects, they begin during treatment and continue beyond the end of treatment. Late effects, in contrast, appear months to years after the completion of treatment. Since tissue damage noted during or at the end of therapy may remain stable or become progressive, late effects refer specifically to these unrecognized toxicities that are absent or subclinical at the end of therapy but manifest later as a result of growth, development, increased demand, or aging. These can be due to any of the following factors: developmental processes; the failure of compensatory mechanisms with the passage of time; or organ senescence. Compensatory mechanisms that initially maintain the function of injured organs may fail over time or with organ senescence. Persistent symptoms differ from late effects of treatment because they begin during treatment and continue following treatment rather than appearing months to years after the completion of treatment (Kolb and Poetscher 1997). Some researchers classify cognitive problems, fatigue, lymphedema, and peripheral neuropathy as persistent symptoms. Patients demonstrating signs or symptoms of late or long-term effects may have to undergo major adjustments to a lifestyle for which they are unprepared (Loescher et al. 1989; Welch-McCaffrey et al. 1989; Herold and Roetzheim 1992; Marina 1997; Aziz 2002).

Late effects of cancer treatment occur because effects of therapy on maturing organs may become manifest only with time or with the unmasking of hitherto unseen injury to immature organs by developmental processes (Schwartz 1999; Aziz 2002). The study of late effects, originally within the realm of pediatric cancer, is now germane to cancer survivors at all ages as concerns may continue to surface throughout the life cycle. These concerns underscore the need to follow up and screen survivors of cancer for toxicities in order to prevent or ameliorate these problems (Aziz 2002; Aziz and Rowland 2003).

## 19.1 Prevalence

The number of cancer survivors in the United States has risen steadily over the past three decades for all cancers combined. In 1971, 3 million people were living with cancer, representing approximately 1.5% of the population. Recent estimates suggest that there are now 9.8 million cancer survivors in the United States, representing approximately 3.5% of the population. In the absence of other competing causes of death, current figures indicate that for adults whose cancer is diagnosed today, 64% can expect to be alive in 5 years; this is up from 50% estimated for those with a cancer diagnosis during 1974 through 1976. Among children, 79% of childhood cancer survivors will be alive at 5 years and nearly 75% at 10 years, compared with 56% alive 5 years after diagnosis during 1974 through 1976. Of child and adult survivors, 14% had a cancer diagnosis 20 or more years ago. More women than men are survivors, even though more males than females receive a cancer diagnosis annually. Men have a higher proportion of lung cancer, for which survival is poor, whereas women have higher proportions of readily detectable and treatable cancers (e.g., breast, gynecologic). Additionally, women generally have a lower all-cause mortality rate than men. Of the prevalent cancer population, the largest constituent group is breast cancer survivors (22%), followed by survivors of prostate cancer (17%), colorectal cancer (11%) and

gynecologic cancer (10%). Cancer is a disease associated with aging. Sixty percent of all newly diagnosed cancers occur among people aged 65 or older, and most survivors (61%) are aged 65 or older. Researchers currently estimate that one of every six persons over the age of 65 is living with a history of cancer. Thirty-three percent of cancer survivors are between the ages of 40 and 64, 5% are 20–39 years of age, and less than 1% are 19 or younger (Aziz 2002; Aziz and Rowland 2003; Rowland 2004).

Fitzhugh Mullan, a physician diagnosed with and treated for cancer himself, first described cancer survivorship as a concept (Mullan 1985). Definitional issues for cancer survivorship encompass three related aspects. First, who is a cancer survivor? Philosophically, anyone who has been diagnosed with cancer is a survivor, from the time of diagnosis to the end of life (EOL) (Aziz 2002; Aziz and Rowland 2003). Caregivers and family members are also included within this definition as secondary survivors. Second, what is cancer survivorship? Mullan described the survivorship experience as similar to the seasons of the year. He recognized three seasons or phases of survival: acute (extending from diagnosis to the completion of initial treatment, encompassing issues dominated by treatment and its side effects); extended (beginning with the completion of initial treatment for the primary disease, remission of disease, or both; dominated by watchful waiting, regular follow-up examinations and, perhaps, intermittent therapy); and permanent survival (not a single moment; evolves from extended disease-free survival when the likelihood of recurrence is sufficiently low). An understanding of these phases of survival is important for facilitating an optimal transition into and management of survivorship. Third, what is cancer survivorship research? Cancer survivorship research seeks to identify, examine, prevent, and control adverse cancer diagnosis and treatment-related outcomes (such as late effects of treatment, second cancers and quality of life (QOL) ), to provide a knowledge base regarding optimal follow-up care and surveillance of cancer survivors, and to optimize health after cancer treatment (Aziz 2002; Aziz and Rowland 2003).

Consistent with the shift in our perceptions of cancer as a chronic disease, new perspectives and an emerging body of scientific knowledge must now be incorporated into Mullan's original description of the survivorship experience (Aziz and Rowland 2003). Mullan's comparison of cancer survivorship with seasons of the year had implied that the availability and widespread use of curative and effective treatments would lead to a low likelihood of recurrence and longer survival times. However, the potential impact of late and long-term adverse physiologic and psychosocial effects of treatment was not described. In addition, further advances in survivorship research over the past few years have necessitated the incorporation of other emerging concepts into the evolving paradigm of cancer survivorship research (Aziz 2002; Aziz and Rowland 2003). These include: the key role of lifestyle and health promotion in ameliorating adverse treatment and disease-related consequences; the impact of comorbidities on a survivors health status and their possible interaction with risk for or severity of late effects; the effect of cancer on the family; and the need for incorporating a developmental and life-stage perspective in order to facilitate optimally a cancer patient's journey into the survivorship phase. A developmental or life-stage perspective is particularly important as it carries the potential to affect and modify treatment decisions, the intensity of post-treatment follow-up care, the risk and severity of adverse sequelae of treatment, and the need for or use of technology (e.g., sperm banking) depending on the survivors age at diagnosis and treatment) (Aziz 2002; Aziz and Rowland 2003).

## 19.2 Survivorship as a Scientific Discipline

The creation of the Office of Cancer Survivorship Program at the U.S. National Cancer Institute (NCI) in 1996 helped to highlight the key importance of cancer survivorship as a research area in its own right, and an integral part of the cancer prevention and control spectrum. Research related to cancer survivors, pertaining to the amelioration or management of adverse late or long-term sequelae of cancer and its treatment, the prevention, control, or management of sources of morbidity, and enhanced length and quality of survival, is a burgeoning area of interest among investigators, practitioners, survivor advocacy groups, and policy makers. Several highly successful research initiatives have been released over the past 8 years by the Office of Cancer Survivorship in order to lay the foundation for and stimulate growth in key areas of cancer survivorship research. The large numbers of successful investigator-initiated grant submissions addressing cancer survivorship relevant research questions who pass through the same stringent scientific criteria for peer review as other grants at the National Institutes of Health (NIH) bear testament to the continued evolution and growth of this scientific discipline, as do the increasing numbers of peer reviewed manuscripts in leading national and international oncologic, medical, psychosocial, and health-related journals.

## 19.3 Prevention

The study of cancer prevention focuses on populations who are most at risk of a potential malignancy. Some populations are at such high risk of cancer that more aggressive treatment interventions may be advocated. Examples include patients with the familial polyposis syndromes or who carry BRCA-1 or BRCA-2 genes. In these instances, early surgical intervention or intense follow up is warranted.

The risk of colorectal cancer approaches 100% in those that suffer from familial polyposis syndromes. Therefore, it is the standard of care to recommend a total proctocolectomy, leaving the patient with either a permanent ileostomy or an ileal pouch anastamosis to the anus. There are many QOL considerations for each approach, such as the multifaceted problems associated with an intestinal stoma, or the risk of multiple bowel movements a day or pouchitis that is associated with an ileal pouch anastamosis. In addition, if the patient opts to leave part or the entire rectum in place, the patient must endure frequent proctoscopic examinations throughout life and will suffer inherent discomfort, along with the psychological stress of potential rectal cancer in the future. These patients must also face the possibility that there is also the chance for tumor occurring somewhere else in the alimentary tract, most notably the duodenum, and upper endoscopy is frequently recommended.

Hereditary breast or ovarian cancer is a second common example wherein a negative cancer survivorship outcome may be manifested in a setting that has generally been considered part of cancer prevention. Patients who are at higher risk must weigh the risks of

BRCA-1 and BRCA-2 testing. If positive, patients may opt for prophylactic bilateral mastectomies, with or without reconstruction. Women must also consider bilateral oophorectomy related to the high risk of ovarian cancer (10–60% increased risk). Adverse medical, physiological, or psychological outcomes may be observed. For example, the impact of knowing that one is at a higher risk of cancer, the potential surgical procedure and its effect on self-image, the effect of suffering from possible lymphedema even without a lymph node dissection, and finally, the outcomes associated with undergoing early menopause, infertility, and concern for future generations.

## 19.4 Smoking Cessation

An understudied but significant issue for cancer survivors is smoking cessation. Changes in diet, tobacco use, and exercise may lead to better physical and emotional QOL for cancer survivors (Demark-Wahnefried et al. 2005). Despite this, recent studies suggest that many cancer survivors do not change their smoking, diet, and exercise behaviors after diagnosis (Bellizzi et al. 2005; Caan et al. 2005; Coups and Ostroff 2005). Notably, nearly a third of patients continue or resume smoking after a cancer diagnosis (Evangelista et al. 2003; Ostroff et al. 1995; Chan et al. 2004) or are regularly exposed to secondhand smoke (Evangelista et al. 2003).

Reasons why smokers continue to smoke after a diagnosis of cancer is complex. Smokers may feel ambivalent to quit when they have already had the greatest perceived risk of smoking-cancer (especially lung cancer) (Weinstein et al. 2004). They also may feel it is the most difficult time to quit related to the stress of having a new diagnosis of cancer, undergoing difficult treatments, and continued follow-ups. Unfortunately, many smokers may not perceive the risks of continuing to smoke or may choose to ignore them.

Smoking during treatment for cancer may delay surgery, impair wound healing, cause lung infections, and contribute to other co-morbidities. Furthermore, continuing to smoke may have serious negative effects on cancer survivors' general health and QOL – a significant concern for this population, in which there is a high rate of survivorship. In addition to the concerns about secondary cancers and other health issues caused by tobacco use, smoking during cancer treatment has been shown to reduce long-term survival of breast cancer and to increase the possibility of developing distant metastases (Nguyen et al. 2003). Smoking in prostate cancer patients treated with radiation has been linked to lower survival rates (Pahlajani 2004) and to higher genitourinary and gastrointestinal toxicity (Pahlajani 2006).

Those diagnosed with head, neck, or lung cancers – cancers clearly linked to tobacco use – are most likely to quit smoking after a diagnosis (Schnoll et al. 2003). Researchers have found that location and severity of disease (Ostroff et al. 1995), alcohol use, and previous quit attempts (Chan 2004) affect patients' likelihood of stopping smoking. For example, Ostroff and colleagues (1995) found that head and neck cancer patients with less severe disease were more likely to continue smoking following diagnosis. While health concerns may inspire someone to quit, social support and physician encouragement play a

big part in the process (Duncan et al. 1992; Gilpin et al. 1993; Shoham et al. 2006, 2007). Furthermore, cancer patients may have special issues to be considered, such as physical mobility, depression, and possible pharmacological interactions in nicotine replacement therapy (Gritz et al. 2006). Since research has revealed a link between depression, anxiety, and smoking in the general population (Tsoh et al. 2000; Morrell and Cohen 2006), cessation interventions for cancer patients must be especially sensitive to this in a group dealing with serious disease.

Smoking during treatment for any type of cancer, even those with cancers that have less linkage to tobacco, can lead to significant health complications and can delay certain procedures, such as surgery. Smoking before and after surgery can have negative effects on wound healing (Warner 2007) and breast reconstruction after mastectomy may be delayed due to cigarette smoking (Krueger and Rohrich 2001). Basic science research reveals that nicotine affects the efficacy of chemotherapeutic drugs, including doxorubicin, which is used to treat breast cancer (Zhou et al. 2007). In addition, smoking along with radiation therapy to the breast may contribute to the development of second cancers of the lung (Ford et al. 2003). Smoking is related to higher prostate cancer mortality (Gong et al. 2008) and in prostate cancer patients who undergo radiation therapy in particular (Pantarotto et al. 2006). Furthermore, smoking while undergoing chemotherapy may contribute to increased pulmonary infections (Chelghoum et al. 2002).

## 19.5 Acute Effects of Treatment

Acute effects of treatment are those that occur during and soon after treatment. Patients suffer many side effects that need to be addressed throughout their course of therapy. Supportive care in the acute setting is focused on known side effects of treatments and, if possible, pre-emptively treating these problems. Surgical, radiation therapy, and chemotherapy techniques continue to make significant improvements that lead to better QOL for cancer survivors. For example, minimally invasive surgical techniques may reduce pain and post operative rehabilitation. Use of high dose brachytherapy for some sarcoma patients can simplify therapy such that patients are less isolated during these treatments. Oral agents (e.g., 5-FU) enable a much simpler and less uncomfortable administration of chemotherapy for colorectal cancer patients.

### 19.5.1 Chemotherapy

There are many acute systemic effects related to chemotherapy. Presently, many of these can be adequately treated with medications to reduce the toxicity associated with current chemotherapy regimens. Gastrointestinal complications, such as nausea, vomiting, and diarrhea, have historically been a major difficulty related to chemotherapy, as well as immunotherapy. They may be effectively treated with multiple medical strategies.

*Nausea and Vomiting*. Certain chemotherapeutics carry higher risks of nausea and vomiting, including doxorubicin, cisplatin, iphosphamide, and dacarbazube. Other risk factors may include chronic alcohol use, female gender, and a history of poor control of nausea and vomiting (Bartlett and Koczwara 2002). While persistent difficulties throughout therapy can lead to altering chemotherapy dosing or discontinuing treatments, initiating antiemetics with treatments can frequently control symptoms. In fact, the use of dexamethasone along with 5-HT3 antagonists has been shown to completely control acute emesis in approximately 85–90% of patients (Bartlett and Koczwara 2002). For patients at moderate risk, dexamethasone alone can control emesis in 90% of patients. Other anti-emetics that can be useful in this setting are metoclopromide, prochorperazine, and benzodiazepines.

*Asthenia* is a problem with multiple potential etiologies (Von Hoff 1998). If related to anemia, multiple strategies can be attempted, the most straightforward being transfusional therapy. This is most useful for patients who have extremely low hemoglobin or those that are unresponsive to other treatments. Treatment with recombinant human erythropoietin, three times a week or weekly, has been shown to have response rates of 50–60% (Gordon 2002). Darbepoetin alfa, a newer erythropoiesis-stimulating protein, can be dosed weekly or every other week, and may show improved response rates (Gordon 2002). Increased hemoglobin levels are correlated with an improvement in symptoms of fatigue. While there are other mechanisms related to fatigue (i.e., other medical problems such as congestive heart failure, depression, hormonal abnormalities, or cytokine production), future studies will help to discern other approaches to this problem.

*Anorexia and Cachexia* are frequently mentioned along with asthenia based on potentially similar pathophysiologies (Von Hoff 1998). Poor appetite and wasting frequently co-exist in a patient, either causally or due to similar etiologic mechanisms. Anorexia and cachexia are problems not only for patients, but are also stressful for families that watch loved ones become thinner and less energetic. Cachexia consists of a constellation of metabolic and symptomatic changes. These include a reduction in lean body tissues and fatty tissues, hypoglycemia, hypercalcemia, as well as asthenia and anorexia. Progressive weight loss is a common problem faced by cancer patients and is responsible not only for a reduced QOL and poor response to chemotherapy, but also a shorter survival time as compared to patients with comparable tumors who do not suffer from weight loss (Tisdale 1999; Hess et al. 2007). Medications such as megestrol acetate (Splinter 1992; Downer et al. 1993; Mantovani 1995; Neri et al. 1997; De Conno et al. 1998; Mantovani et al. 1998), medroxyprogesterone acetate (Splinter 1992; Downer et al. 1993; Mantovani 1995; Neri et al. 1997; De Conno et al. 1998; Mantovani et al. 1998), and potentially thalidomide (Bruera et al. 1999) can be helpful for some patients, but further study is warranted.

*Immunosuppression and Risk of Infection* play an overwhelming role in a patient's QOL. The risk of infection mandates lifestyle changes for patients and as well as for families to protect their loved ones. This may include minimal contact with people, careful and frequent washing of hands, and the use of masks. Judicious use of antibiotics, along with cytokine therapy to enhance white blood cell counts, lessens infection (ASCO 1994). Granulocyte colony-stimulating factor has also been shown to reduce hospitalization and antibiotic therapy (Garcia-Carbonero et al. 2001). Patients who maintain or achieve adequate white blood cell counts have an improved QOL and may be better able to tolerate of chemotherapy treatment (Jones et al. 1996), although this is not always seen (Steward et al. 1998).

### 19.5.2 Surgery

Operative therapies are frequently curative and QOL-improving in a number of cancers (i.e., breast cancer, colon cancer, and melanoma). Improvement in QOL may be related to the removal of a potentially painful or problematic tumor-related wound (e.g., from a breast or skin cancer), or the removal of a tumor in the colon that had been causing bleeding or obstruction. For a tumor that is unlikely to be cured via an operation, such as esophageal or gastric cancers, surgical approaches have been shown to improve the ability to swallow for many patients, which can in turn improve QOL (Branicki et al. 1998). Surgical procedures can also lead to multiple acute morbidities. Risks associated with each surgical procedure must be carefully considered, as complications will still occur even in the most fastidious care, especially if the patient is debilitated related to the cancer or underlying conditions. First, surgical morbidity may include complications unrelated to the surgical site, such as pneumonia, deep venous thrombosis, ileus, and heart failure. With meticulous care, these can often be avoided. Related to the procedure itself, pain is a major issue that occurs in the postoperative setting, and may persist. Epidural, patient controlled analgesia, and local anesthetic pumps may improve pain control and ultimate outcomes. Improved outcomes can be seen as less impairment of pulmonary, bowel, mental functions, nutritional status, coagulation, immune function, as well as reducing the risk of chronic pain (Karanikolas and Swarm 2000; Reid 2001; Fotiadis et al. 2004). Acceptance of disfigurement and life-style changes are most pronounced in the immediate postoperative setting. For example, for many patients undergoing surgery related to colorectal cancer, the shock of a permanent stoma may be overwhelming. In fact, this may be the overwhelming issue of concern for patients with a new cancer, possibly even leading to delay in treatment (Cohen et al. 1997). Recent evidence suggests that QOL problems related to ostomies may diminish with time, but nevertheless remain a significant factor in the postoperative setting (Krouse et al. 2004).

Wound complications must always be considered in the setting of cancer-related surgical procedures. This is certainly true of lymph node dissections; wound problems have been noted to be 47% for axillary node and 71% after inguinal node dissections (Serpell et al. 2003). Seromas and infections may take from several weeks to months to heal. Lymphatic leaks may necessitate procedures to isolate the offending lymphatic vessel. Therefore, when considering any surgical procedure, whether for curative or palliative intent, these issues must be discussed prior to the operation. As new innovations are utilized, outcomes will continue to improve for surgical patients.

### 19.5.3 Radiation Therapy

There are many acute effects of radiation therapy. These are based on the location of the treatment. If treatment is directed into the peritoneal cavity, the most likely problems will be related to cramping, abdominal pain, and diarrhea. Fatigue is also a common problem for patients undergoing radiation therapy (Irvine et al. 1998). Radiation of the

upper aerodigestive tract may lead to edema and inability to swallow. Localized skin irradiation can lead to painful burns. These symptoms may be very difficult to control initially, but frequently improve with time. Newer treatments are being tested to reduce the acute effects of radiation, such as silver leaf nylon dressings for perineal irradiation (Vuong et al. 2004). Other promising treatments include glutamine to protect against radiation-induced injury (Savarese et al. 2003), and hyperbaric oxygen therapy for radiation-induced osteoradionecrosis, soft tissue necrosis, cystitis, proctitis (Bui et al. 2004), or breast skin burns (Borg et al. 2001). Other treatments for skin damage, such as transparent, hydrocolloid, and hydrogel dressings, have demonstrated some benefit, as have sucralfate cream and corticosteroid cream. Aloe vera may be beneficial and has no known side effects (Wickline 2004).

## 19.6 Long-Term and Late Effects of Cancer Treatment

Most cancer treatments carry substantial risk of adverse long-term or late effects, including neurocognitive problems, premature menopause, cardiac dysfunction, sexual impairment, chronic fatigue, pain, and second malignancies for both adult and childhood cancer survivors (Schwartz et al. 1993; Kolb and Poetscher 1997; Aziz 2002; Aziz and Rowland 2003). One fourth to one third of breast and lymphoma survivors who receive chemotherapy may develop detectable neurocognitive deficits (Cimprich 1992; Ganz 1998; Brezden et al. 2000; Ahles et al. 2002). Late clinical cardiotoxicity, often life threatening, may occur in 5–10% of long-term pediatric cancer survivors even 5–10 years after therapy (Simbre et al. 2001).

Late effects of radiotherapy and chemotherapy are related to organ dysfunction and impact a patient's life by altering functional abilities (Table 19.1). The lifestyle changes required by patients are associated with specific drugs used in the treatment regimen, which are frequently prescribed dependent on the location of a solid tumor. Combinations of chemotherapy and radiation therapy have a higher incidence of late effects of treatment (Aziz 2002; Aziz and Rowland 2003). In addition, long-term effects on organ systems may lead to mortality. This has been noted by investigators to account for one-fourth of late deaths of cancer survivors. The most common causes of late deaths among survivors of pediatric cancer include secondary cancer or cardiac dysfunction (Sklar 1999).

Late effects can be classified further as: (a) system specific (i.e., damage, failure or premature aging of organs, immunosuppression or compromised immune systems, and endocrine damage); (b) second malignant neoplasms (i.e., increased risk of a certain cancer associated with the primary cancer and a second cancer associated with cytotoxic or radiological cancer therapies); (c) functional changes (lymphedema, incontinence, pain syndromes, neuropathies and fatigue); (d) cosmetic changes (i.e., amputations, ostomies and skin and hair alterations); and (e) associated comorbidities (i.e., osteoporosis, arthritis, scleroderma and hypertension) (Aziz 2002; Aziz and Rowland 2003). The risk of a recurrence of the primary malignancy, while not a late effect, is also ever present and affects surveillance, monitoring and post-treatment follow-up management decisions.

**Table 19.1** Possible late effects of radiotherapy and chemotherapy

| Organ system | Late effect/sequelae of radiotherapy | Late effect/sequelae of chemotherapy | Chemotherapeutic drugs responsible |
|---|---|---|---|
| Bone and soft tissues | Short stature; atrophy, fibrosis, osteonecrosis | Avascular necrosis | Steroids |
| Cardiovascular | Pericardial effusion; pericarditis; CAD | Cardiomyopathy; CCF | Anthracylines |
| | | | Cyclophosphamide |
| Pulmonary | Pulmonary fibrosis; decreased lung volume | Pulmonary fibrosis; interstitial pneumonitis | Bleomycin |
| | | | BCNU |
| | | | Methotrexate |
| | | | Adriamycin |
| Central nervous system | Neuropsychological deficits; structural changes; hemorrhage | Neuropsychological deficits, structural changes; hemiplegia; seizure | Methotrexate |
| Peripheral nervous system | | Peripheral neuropathy; hearing loss | Cisplatin |
| | | | Vinca alkaloids |
| Hematological | Cytopenia, myelodysplasia | Myelodysplastic syndromes | Alkylating agents |
| Renal | Decreased creatinine clearance; hypertension | Dec. creatinine clearance; Inc. creatinine; Renal F Delayed Renal F | Cisplatin |
| | | | Methotrexate |
| | | | Nitrosoureas |
| Genitourinary | Bladder fibrosis, contractures | Bladder fibrosis; Hemorrhagic cystitis | Cyclophosphamide |
| Gastrointestinal | Malabsorption; stricture; abnormal LFT | Abnormal LFT; Hepatic fibrosis; cirrhosis | Methotrexate |
| | | | BCNU |
| Pituitary | Growth hormone deficiency; pituitary deficiency | | |
| Thyroid | Hypothyroidism; nodules | | |

(continued)

**Table 19.1** (continued)

| Organ system | Late effect/sequelae of radiotherapy | Late effect/sequelae of chemotherapy | Chemotherapeutic drugs responsible |
|---|---|---|---|
| Gonadal | Men: risk of sterility; Leydig cell dysfunction Women: ovarian failure; early menopause | Men: sterility Women: sterility, premature menopause | Alkylating agents Procarbazine |
| Dental/oral health | Poor enamel and root formation; dry mouth | | |
| Opthalmological | Cataracts; retinopathy | Cataracts | Steroids |

Adapted from Aziz (2002), Aziz and Rowland (2003), and Ganz (1998)

## 19.7 Generalizations

Certain types of late effects can be anticipated from exposure to specific therapies, age of the survivor at the time of treatment, combinations of treatment modalities and dosage administered (Aziz 2002). Susceptibility differs for children and adults. Generally, chemotherapy results in acute toxicities that can persist, whereas radiation therapy leads to sequelae that are not immediately apparent. Combinations of chemotherapy and radiation therapy are more often associated with late effects. Risk of late death from causes other than recurrence is greatest among survivors treated with a combination of chemotherapy and radiotherapy. Toxicities related to chemotherapy, especially those of an acute but possibly persistent nature, can be related to proliferation kinetics of individual cell populations because these drugs are usually cell-cycle dependent. Organs or tissues most susceptible have high cell proliferation rates and include the skin, bone marrow, gastrointestinal mucosa, liver and testes. The least susceptible organs and tissues replicate very slowly or not at all and include muscle cells, neurons and connective tissue. However, neural damage may be caused by commonly used chemotherapeutic drugs such as methotrexate, vinca alkaloids and cytosine arabinoside, bone injury may be caused by methotrexate, and cardiac sequelae can occur after treatment with adriamycin. Injuries in tissues or organs with low repair potential may be permanent or long lasting.

*Issues Unique to Certain Cancer Sites*. The examination of late effects for childhood cancers such as leukemia, Hodgkin's lymphoma and brain tumors have provided the foundation for this area of research. A body of knowledge on late effects of radiation and chemotherapy is also now appearing for adult cancer sites such as breast cancer. For example, neurocognitive deficits that may develop after chemotherapy for breast cancer are an example of a late effect that was initially observed among survivors of childhood cancer receiving cranial irradiation, chemotherapy or both (Kreuser et al. 1988; van Dam et al. 1998; Ahles et al. 2002; Aziz 2002; Aziz and Rowland 2003). Late effects of bone marrow

transplantation have been studied for both adult and childhood cancer survivors as have sequelae associated with particular chemotherapeutic regimens for Hodgkin's lymphoma and breast cancer (Sankila et al. 1996; Schwartz 1999; Greendale et al. 2001; Aziz 2002). The side effects of radiotherapy, both alone and with chemotherapy, have been reported fairly comprehensively for most childhood cancer sites associated with good survival rates. Most cancer treatment regimens consist of chemotherapy in conjunction with surgery or radiation, and multidrug chemotherapeutic regimens are the rule rather the exception. As such, the risk of late effects must always be considered in light of all other treatment modalities to which the patient has been exposed.

*Special Considerations Related to Age at Diagnosis*. Long-term cancer survivors are faced with different effects of treatment depending on the age at diagnosis. Children face growth, neurocognitive, and hormonal imbalance related to cancer treatment. Young adults frequently face issues such as reproductive function and risks for second cancers. Middle age patients face problems related to chronic disease due to the effects of treatment and early menopause, and older patients frequently suffer from additional co-morbidities, making long-term complications potentially more deleterious (Aziz 2002; Aziz and Rowland 2003).

*Special Considerations when Primary Diagnosis and Treatment Occurs in Childhood*. Cancer therapy during childhood may interfere with physical and musculoskeletal development (Blatt et al. 1988; Furst et al. 1989; Sklar et al. 1993; Ogilvy-Stuart et al. 1994; Didi et al. 1995), neurocognitive and intellectual growth (Ochs et al. 1991; Haupt et al. 1994), and pubertal development (Kreuser et al. 1988). These effects may be most notable during the adolescent growth spurt. Prevention of second cancers is also a key issue (Mullan 1985; Aziz 2002).

Some late effects of chemotherapy may assume special importance depending on the adult patient's age at the time of diagnosis and treatment (Schwartz 1999; Aziz 2002; Aziz and Rowland 2003). Diagnosis and treatment during the young adult or early reproductive years may call for a special cognizance of the importance of maintaining reproductive function and the prevention of second cancers (Shahin and Puscheck 1998). Cancer patients who are diagnosed and treated around 30–50 years of age may need specific attention for premature menopause, issues relating to sexuality and intimacy, the use of estrogen replacement therapy, prevention of neurocognitive, cardiac and other sequelae of chemotherapy, and prevention of coronary artery disease and osteoporosis (Herold and Roetzheim 1992; Marina 1997; Schwartz 1999; Aziz 2002). Sexual dysfunction may persist after breast cancer treatment and may include vaginal discomfort, hot flashes, and alterations in bioavailable testosterone, luteinizing hormone and sex hormone binding globulin (Ganz et al. 2000; Greendale et al. 2001). Menopausal symptoms such as hot flashes, vaginal dryness and stress urinary incontinence are very common in breast cancer survivors and cannot be managed with standard estrogen replacement therapy in these patients. The normal life expectancy of survivors of early-stage cancers during these years of life underscores the need to address their long-term health and QOL issues (Herold and Roetzheim 1992; Marina 1997; Schwartz 1999).

Although older patients (65 years of age or more) bear a disproportionate burden of cancer, advancing age is also associated with increased vulnerability to other age-related health problems, any of which could affect treatment choice, prognosis and survival.

Hence, cancer treatment decisions may have to consider preexisting or concurrent health problems (comorbidities). Measures that can help to evaluate comorbidities reliably in older cancer patients are warranted. Little information is available on how comorbid age-related conditions influence treatment decisions and the subsequent course of cancer or the comorbid condition. It is also not known how already compromised older cancer patients tolerate the stress of cancer and its treatment and how comorbid conditions are managed in light of the cancer diagnosis (Yancik et al. 2001; Aziz 2002).

## 19.8 Physiologic Sequelae of Cancer and Its Treatment

*Second Cancers*. Second cancers may account for a substantial number of new cancers. A second primary cancer is associated with the primary malignancy or with certain cancer therapies (e.g., breast cancer after Hodgkin's lymphoma, ovarian cancer after primary breast cancer) (Ho and Frei 1971; Zimm et al. 1983; Meadows et al. 1985; Hawkins et al. 1987; Hildreth et al. 1989; Sankila et al. 1996). Commonly cited secondary malignancies include: (a) approximate 20% risk of myelodysplasic syndromes, acute leukemia, and non-Hodgkin's lymphoma due to the chemotherapy combinations for Hodgkin's lymphoma (alkylating agents and podophyllotoxins); (b) solid tumors such as breast, bone, and thyroid cancer in the radiation fields in patients treated with radiotherapy; and (c) bladder cancer after cyclophosphamide. Secondary solid malignancies have been associated with chemotherapy treatments Hodgkin's lymphoma up to 20 years after therapy (Foss et al. 2002). Secondary cancers may also include risks of squamous cell cancer of the skin and sarcoma in radiation fields, such as with breast cancer patients. Within 20 years, survivors of childhood cancer have an 8–10% percent risk of developing a second cancer (Draper et al. 1986). This can be attributed to the mutagenic risk of both radiotherapy and chemotherapy, which is further compounded in patients with genetic predispositions to malignancy. The risk of a second cancer induced by cytotoxic agents is related to the cumulative dose of drug or radiotherapy (Herold and Roetzheim 1992; Marina 1997; Schwartz 1999; Aziz 2002).

The risk of malignancy with normal aging may be a result of cumulative cellular mutations. The interaction of the normal aging process and exposure to mutagenic cytotoxic therapies may result in an increased risk of second malignancy, particularly after radiotherapy and treatment with alkylating agents and podophyllotoxins. Commonly cited second cancers include leukemia after alkylating agents and podophyllotoxins; solid tumors, including breast, bone and thyroid cancer in radiation fields; and bladder cancer after cyclophosphamide. Second cancers may also occur in the same organ site (e.g., breast, colorectal); thus, there is definite need for continued surveillance (Herold and Roetzheim 1992; Marina 1997; Schwartz 1999; Aziz 2002).

The risk of secondary malignancy can have long-term psychological impact for patients, as well as overwhelming life changes should a second malignancy occur. In efforts to prevent secondary cancers, treatments may be offered that are associated with other risks on patient QOL. For example, oophorectomy in a breast cancer patient may lead to early menopause, and the risks associated with surgery. If anti-hormonal treatments are implemented, such as

tamoxifen to prevent another breast cancer, risks include cardiac toxicity, thrombotic events, or endometrial cancer. These risks must be considered by practitioners and patients prior to initiating treatment. Some patients may initiate alternative medical approaches, which frequently carry unknown risks and unclear benefits (Aziz 2002a).

*Neurocognitive Function*. Most data related to the long-term neurocognitive effects of treatments have focused on brain tumors, especially in children. This may be related to surgery, radiation therapy, or chemotherapy. Chemotherapeutics have been associated with long-term neurocognitive deficits for non-CNS tumors (Hess and Insel 2007). While in adults this has been primarily investigated among breast cancer survivors (Schagen et al. 1999; Ahles et al. 2002; Schagen et al. 2002, Castellon et al. 2004), neurocognitive decline is likely to occur in many patients who have been treated with chemotherapeutics (Ahles et al. 2002). For breast cancer survivors, neurocognitive compromise is most profound when tamoxifen was added to chemotherapeutic regimens. (Castellon et al. 2004) Despite the preliminary state of this field of research, studies have consistently demonstrated a decline in verbal memory, executive function, and motor function among cancer patients (Anderson-Hanley et al. 2003). Future research efforts should further investigate these problems to better understand these neurocognitive changes so that preventive or treatment strategies can be developed.

*Gastrointestinal Dysfunction*. Radiation therapy has been found to cause fibrosis and stricturing along the alimentary tract, and is caused by both brachytherapy (Hishikawa et al. 1986) and external beam (DeCosse et al. 1969; Palmer and Bush 1976). This problem has also been noted with the utilization of photodynamic therapy (McCaughan et al. 1996; Overholt and Panjehpour 1996). The alimentary tract is at risk in the primary or adjuvant treatment of esophageal or rectal tumors; small bowel injury may also occur in the treatment of other intraabdominal processes, such as pre- or post-operative radiation therapy for retroperitoneal sarcomas. Large or small bowel strictures can lead to obstruction, the symptoms of which include nausea, vomiting, pain, and bloating. Esophageal strictures can also cause difficulty or inability to swallow. For esophageal or colonic strictures, endoscopic approaches utilizing stents may be beneficial. This approach is not applicable for most of the small bowel, which may necessitate an operative approach, usually either a bypass or resection. Some surgeons utilize minimally invasive laparoscopic techniques, although this may not be possible if there are abundant adhesions. Long-term gastrointestinal function is also common among patients suffering from gynecologic cancers. One common example is related to radiation enteritis in the setting of radiation therapy for cervical cancers. In addition, 5–51% of patients with ovarian cancers present with a bowel obstruction, either as an initial presentation or as a recurrence (Davis and Nouneh 2001). Treatment in both of these settings may be difficult and there is no obvious standard of care.

*Pulmonary Dysfunction*. Pulmonary dysfunction may be related to major resections, radiation injury, or chemotherapeutic injury. This may be enhanced if the patient has preexisting pulmonary problems. The incidence of lung injury after breast irradiation and after lumpectomy is minimal, but not irrelevant (Kimsey et al. 1994; Dolsma 1997; Theuws et al. 2000). In the context of the large number of women undergoing lumpectomy and radiation therapy as a primary treatment for breast cancer, this problem may be significant, and even more so among women who have underlying lung disease (Theuws et al. 1998). There are many chemotherapeutic agents that can cause pulmonary injury. Bleomycin may

be the most notorious, although others include cyclophosphamide, mitomycin, carmustine, and methotrexate. Whenever using these medications, this complication should be anticipated. Therefore, it is recommended to assess pulmonary function tests at baseline and every three months while on therapy, and carefully follow patients for signs of impending problems such as dyspnea and hypoxia (Ignoffo 1998). Finally, major resections, such as pneumonectomy, may leave patients debilitated and unable to carry out normal functions.

*Cardiac Dysfunction*. Injury to the heart from chemotherapy, most notably doxorubicin, or from chest wall irradiation is a known risk of cancer treatment. Clinical manifestations of anthracyclins include reduced cardiac function, arrhythmia, and heart failure (Lipshultz et al. 1991, 2002; Ganz 1998). Radiation therapy can lead to valvular damage, pericardial thickening, ischemic heart disease (Lipshultz et al. 1991, 2002) and a decreased ejection fraction (Mukherjee et al. 2003). These may have long-term impact on a patient's QOL and ability to lead a reasonably active life. Protective agents, such as dexrazoxane or amifostine, may be given during chemotherapy to lessen the risk of cardiac damage. Other agents that have been shown to be protective for doxorubicin therapy are under investigation. (Olson et al. 2003; Oliveira et al. 2004) Consideration of myocardial injury at the time of radiotherapy and may be lessened by breathing techniques (Sixel et al. 2001). Despite these preventive measures, heart damage is not completely avoidable in patients undergoing these treatments, and may cause considerable long-term morbidity.

*Endocrine Dysfunction*. There are multiple potential endocrine problems related to cancer prevention and treatment (Sklar 1999). One common disorder is related to thyroid cancer, whereby total thyroidectomy will necessitate life-long thyroid replacement therapy. In the case of pancreatic surgery, deficiencies of pancreatic hormones, such as insulin, are treatable, yet they may severely alter a patient's life. This may include the need to control diet, use oral hypoglysurics, or prescribe insulin therapy. Perhaps the most common hormonal difficulty is related to the surgical or medical ablation of estrogen in female patients. This will lead to temporary or permanent menopause symptoms. Hot flashes related to tamoxifen can be debilitating and necessitate discontinuing this medication. Tamoxifen blocks estrogen receptors in most hormonal tissues, but is considered a mixed estrogen antagonist/agonist. Other newer medications that block estrogen, either through production or activity, are aromatase inhibitors, megestrol acetate and fulvestrant, which are great cause for optimism for breast cancer patients (Osipo et al. 2004). However, control of symptoms does not also compensate for the loss of hormones with regard to bone loss. Future strategies to prevent this side effect must be considered in the future (Coleman 2004).

Another hormone dysfunction that can severely alter a patient's QOL is related to advanced neuroendocrine tumors. These can include the carcinoid syndrome, glucagonoma, and insulinoma. There are multiple techniques that can limit the effects of these tumors. These include somatostatin, alpha-interferon, ablative techniques (e.g., radiofrequency ablation, hepatic artery chemoembolization) and surgical extirpation) (Krouse 2004). These techniques have shown benefit in multiple instances. The rarity of these tumors makes large prospective studies for these cancers very difficult.

*Intestinal Stomas*. While intestinal stomas will clearly lead to QOL difficulties as described above, some patients will have long-term problems that may continue to affect daily life. Many issues of concern include problems with travel, intimacy, and satisfaction

with appearance. For patients with colostomies and ileostomies, HR-QOL seems to improve with time (Grant 2000), although the evidence is not consistent in all studies (Klopp 1990). Many studies have documented additional issues of concern for ostomates, including sexuality (Hojo et al. 1991; Grunberg 1986; Ofman and Auchincloss 1992; Fazio et al. 1980; Yeager and Van Heerden 1980; Borwell 1997; Sprangers et al. 1995), psychological problems (Thomas et al. 1987; Hurny and Holland 1985; Keyes et al. 1987; Wirsching et al. 1975; Sutherland et al. 1952; Krouse et al. 2007b), and interference with work, recreational and sporting activities (Sprangers et al. 1995; Wirsching et al. 1975; Krouse et al. 2004, 2007a, b). Reasons for continued problems may be due to lack of perioperative teaching, poor stoma placement, stoma related complications such as hernia or prolapse, or lack of a familial or support group assistance. Research is still needed to better understand the nature and cause of many of these problems so that appropriate interventions may be designed.

*Lymphedema*. The long-term effects of lymph node dissection are frequently related to disruption of nerves or lymphatics. Nerve disruption may lead to pain syndromes, numbness, or other effects such as paresthesias (Nagel et al. 2003). There is a large spectrum of presentations of lymphedema from axillary dissection, from arm heaviness to elephantiasis. Reported lymphedema rates are variable but likely around 27% (Beaulac et al. 2002; Golshan et al. 2003; Voogd et al. 2003; Bani et al. 2007). This rate may be as high as 49% for long-term survivors (Petrek et al. 2001). Sentinel node mapping decreases this risk (Haid et al. 2002; Golshan et al. 2003; Schijven et al. 2003; Lucci et al. 2007). It is likely that many patients (38–93%) will also have other limb symptoms such as pain, numbness, poor range of motion, and weakness (Ververs et al. 2001; Engel et al. 2003; Nagel et al. 2003; Schijven et al. 2003). In addition, newer technologies will assist in the early diagnosis of lymphedema, which will also potentially lead to greater numbers of patients diagnosed with this disorder.

Lymphedema is a risk for patients who have radiation therapy to lymph node basins (Erickson et al. 2001; Meric et al. 2002; Bani et al. 2007). This is especially accentuated if there is a previous lymph node dissection in the same basin. Lymphedema may occur early or many years from treatments and is especially true if lymph node basins have been irradiated in addition to node dissections (Kissin et al. 1986; Ryttov et al. 1988). Close observation and prevention must be considered along with treatment approaches, including arm elevation, use of compression stockings, and massage techniques. There are increasing reports of successes with this active treatment approach (Kim et al. 2007; Vignes et al. 2007; Moseley et al. 2007; Hamner and Fleming 2007). Historically, surgical techniques for massive lymphedema have primarily focused on debulking procedures. Liposuction, along with compression therapy, has been reported to be beneficial in this setting (Brorson et al. 2006). In addition, microvascular surgical techniques may provide patients with other options to this debilitating problem (Campisi et al. 2007).

*Pain*. The issue of pain related to cancer and its treatment is quite common and plays an overwhelming role in a patient's life. Nearly 75% of patients with advanced cancer have pain, with most having moderate or greater levels of pain (ACS 2001). Chronic pain may be related to nerve disruption at the time of surgery, tumor-related persistent pain, or other treatment related issues. Frequently, these may not be well described by the patient, or well understood by the medical team. The psychological health of the patient may have

a significant role in the perception of pain, and chronic pain may also severely negatively impact a patient's psychological health (ACS 2001). If a patient has a poor prognosis, pain may be accentuated in comparison to a patient who is in remission.

A focus on pain has brought anesthesia pain specialists, palliative care specialists, and others together to study optimal approaches to the myriad of pain syndromes faced by long-term cancer survivors. Clearly, there are many approaches to the treatment of pain. The etiology, type, intensity, location, and time course of pain, as well as a patient's tolerance to pain, may all impact the choices of care. For example, bone metastases may be treated with the use of opioids, steroids, radiation therapy, radiofrequency ablation, or nerve blockade. Based on a thorough understanding of the pain experienced by the patient, the optimal approach or approaches can be initiated. The recent use of radiofrequency ablation to treat soft tissue pain (Locklin et al. 2004) is a novel attempt to treat pain based on a new technology. The use of complementary non-drug techniques may play an increasing role as they are studied. For example, it has recently been reported that massage therapy is an effective and safe adjuvant therapy for the relief of acute postoperative pain after a major operation (Mitchinson et al. 2007).

*Cosmesis*. Cosmetic problems are noted with many surgical procedures, such as amputations, neck dissections, major facial tumor resections, mastectomies, and placement of intestinal stomas. Scarring can be psychologically debilitating for a patient. Minimally invasive procedures have provided improved cosmetic results. This may include laparoscopic colectomies for colon cancer, with smaller incisions and thus smaller scars and limb-sparing approaches for sarcomas. These approaches are either not applicable or attempted for many surgical procedures due to size of tumor, location of tumor, experience of the surgeon, or inability to achieve surgical objectives such as negative margins.

Cosmetic problems are most notable with head and neck cancers. Surgical treatment, or effects of the tumor itself, may result in facial nerve injury. Major reconstruction may be necessary, and results are most obvious to patients, families, and onlookers. In fact, about 50% of patients who undergo procedures on head and neck cancers feel that this is a moderate to severe problem (List and Bilir 2004). Survival is not diminished with breast-sparing lumpectomies followed by radiation (Fisher et al. 1995). Cosmesis is usually quite good; this is a standard of care for breast cancer patients. For those who choose to have mastectomy, or those for whom a mastectomy is recommended, reconstruction approaches are available to improve one's body image.

Radiation therapy may leave cosmetic results that can be quite disturbing to the patient and family. Cosmetic damage due to radiation may lead to trismus when treating head and neck cancers, or alterations of the breast. While these problems may improve with time, there may be long-term or even lifetime difficulties.

*Phonation*. The ability to speak may be impaired with many head and neck cancers and brain tumors (primary and metastatic). This may be due to recurrent layrngeal nerve invasion or injury during a surgical procedure, laryngectomy, or tongue resections. Recurrent laryngeal injury will lead to a hoarse voice and inability to yell, although this frequently improves with time. Removal of the larynx will leave the patient unable to speak, requiring that a patient communicate by writing or through a device pressed against the submental area. This device gives the patient a robotic voice, which may be unacceptable for many patients. Loss of the tongue will make it difficult or impossible to enunciate words,

although many patients will frequently be able to be understood in conversation. The ability to speak and be understood is a human function that is frequently taken for granted until it is no longer possible.

*Swallowing*. The ability to swallow, imperative for the ability to eat, has significant social impact of a persons QOL (List and Bilir 2004). The inability to swallow and the need for a feeding tube has been noted to be the most important QOL issue among head and neck cancer patients (Terrell et al. 2004). The ability to preserve swallowing function is likely the most important outcome of esophageal surgery. Curative surgery is generally unlikely in the setting of esophageal cancer, but maintaining the ability to eat is an attainable goal by removing the tumor and maintaining gastrointestinal continuity via a surgically created neoesophagus (Branicki et al. 1998).

*Sexual Dysfunction*. Sexual function is important to most people, yet estimates of the prevalence of sexual dysfunction in persons with cancer range from 20 to 90% (Ganz 1998; Varricchio 2000; Ganz 2001; Aziz 2002). Sexuality encompasses a spectrum of issues ranging from how one feels about ones body to the actual ability to function as a sexual being. Sexual dysfunction has been reported as a persistent effect of cancer treatment. Dysfunction may be related to multiple factors, including nerve injury, disfigurement and perceived loss of sexuality, and loss of libido. Pre-existing sexual dysfunction may also be exacerbated by cancer and its treatment (Ganz et al. 1992).

Emotional response to the diagnosis or treatment of cancer can have dramatic effect on sexuality issues (Varricchio 2000). Loss of perceived sexuality, by either the patient or the partner, can lead to loss of sexual interactions that once were active and fulfilling. These perceptions may be related to bodily changes, such as creation of a stoma or removal of a breast. These perceptions can limit a patient's interest in sex and interfere with sexual arousal and satisfaction. Pain or bodily function problems may not allow the patient to relax and enjoy sex. By learning what cancer means to the patient and their loved ones, the clinician can correct misinformation and facilitate the patient's adjustment to the illness (Ell et al. 1988).

Gonadal dysfunction or failure can lead to infertility for both male and female cancer survivors (Sklar 1999; Ganz 2001; Aziz 2002). Recovery of gonadal function will depend on type of therapy (such as radiation therapy or alkylating agents) and dosing. Cryopreservation of sperm should always be considered, and ova if possible.

Nerve injury has implications for both males and females, although these issues have been studied much more for males. Without rehabilitation, approximately 85–90% of men with prostate cancer suffer erectile impotence secondary to surgery or radiation (Stoudemire et al. 1985; McKenna 1995). For pelvic cases in males, surgical strategies employing sharp dissection of the mesorectum will allow visualization of the sympathetic and parasympathetic nerves, thus preserving erectile and ejaculatory function in most cases (Meuleman and Mulders 2003). These strategies should also be employed in women to best preserve sexual function. While radiation therapy has less incidence of nerve injury than surgical interventions, it nevertheless carries this risk (Goldstein et al. 1984).

*Xerastomia*. Dry mouth is a common effect of head and neck irradiation including the parotid nodal field. Improved radiation therapy techniques have focused on lessening the effects of this condition, but this remains a problem for many patients. Patients who suffer from xerastomia frequently must have water nearby at all times. This problem is usually

a lifetime issue for patients (August et al. 1996; Liem et al. 1996; Kosuda et al. 1999; Johansson et al. 2002). While it has been shown that many patients adequately adapt to this problem, it remains a serious morbidity of head and neck irradiation. Salivary flow can be stimulated by the use of cholinergic pharmaceutical preparations. Pilocarpine may lead to symptomatic improvement (Hawthorne and Sullivan 2000), but research is necessary to improve this treatment-related survivorship issue. If these treatments fail, mouthwash and saliva substitutes are secondary options (Nieuw Amerongen and Veerman 2003). Bioactive saliva substitutes and mouthwashes are currently under investigation for application in the clinic. These contain antimicrobial peptides to protect the oral tissues against microbial colonization and to suppress and to cure mucosal and gingival inflammation (Nieuw Amerongen and Veerman 2003).

*Asthenia/Anorexia/Cachexia*. Overwhelming fatigue, loss of appetite, and wasting are difficult issues that affect the long-term as well as the short-term QOL of cancer survivors. Asthenia impacts all phases of life, and therefore must be addressed over time. Severe fatigue is a problem for almost 40% of breast cancer survivors (Servaes et al. 2002). Breast cancer patients who experienced severe fatigue suffered from problems with psychological well-being, functional impairment, sleep disturbance, physical activity, social support, neuropsychological and social functioning as compared with breast cancer survivors who did not have persistent fatigue. Therefore, it is imperative to address all known etiologies, including depression, anemia and drug and alcohol use. For patients who are Hodgkin's lymphoma survivors, one-half of the fatigue cases have psychological distress that may respond to treatment (Loge et al. 2000). Related to chronic anorexia, progestational drugs can somewhat stimulate appetite, food intake, and energy level, they promote weight gain in some patients, and often decrease nausea and vomiting severity; however, pharmacologic treatment of cancer cachexia remains disappointing (Body 1999).

Cachexia is the most common paraneoplastic syndrome of malignancy, causing the death in as many as 20% of patients with cancer (Ottery 1994). While frequently considered for patients undergoing treatment or those near the EOL, these problems may persist among cancer survivors (Body 1999). Patients with advanced or chronic disease may live for years and suffer slow wasting over time. There are few current treatment options.

*Grading of Late Effects*. The assessment and reporting of toxicity, based on the toxicity criteria system, plays a central role in oncology. Grading of late effects can provide valuable information for systematically monitoring the development, progression, or regression of late effects (Trotti 2002). While multiple systems have been developed for grading the adverse effects of cancer treatment (Ganz 1998), there is no current universally-accepted grading system (Aziz 2002; Trotti 2002; Aziz and Rowland 2003). In contrast to the progress made in standardizing the measurement of acute effects, the use of multiple grading systems for late effects hinders the comparability of clinical trials, impedes the development of toxicity interventions, and encumbers the proper recognition and reporting of late effects. The wide adoption of a standardized criteria system can facilitate comparisons between institutions and across clinical trials (Trotti 2002; Aziz and Rowland 2003).

Multiple systems have been developed and have evolved substantially since first introduced more than 20 years ago (Hoeller et al. 2003). Garre and colleagues (Garre et al. 1994) developed a set of criteria to grade late effects by degree of toxicity as follows: grade 0 – no late effect; grade 1 – asymptomatic changes not requiring any corrective measures,

and not influencing general physical activity; grade 2 – moderate symptomatic changes interfering with activity; grade 3 – severe symptomatic changes that require major corrective measures and strict and prolonged surveillance; and grade 4 – life threatening sequelae. A similar system, the Swiss Pediatric Oncology Group (SPOG) grading system, has not yet been validated. The SPOG system also ranges from 0 to 4: grade 0 – no late effect; grade 1 – asymptomatic patient requiring no therapy; grade 2 – asymptomatic patient, requires continuous therapy, continuous medical follow-up, or symptomatic late effects resulting in reduced school, job, or psychosocial adjustment while remaining fully independent; grade 3 –physical or mental sequelae not likely to be improved by therapy but able to work partially; and grade 4 – severely handicapped, unable to work independently (von der Weid 1996).

The NCI Common Toxicity Criteria (CTC) system was first developed in 1983. The most recent version, Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) represents the first comprehensive, multi-modality grading system for reporting both acute and late effects of cancer treatment. Version 3.0 incorporates physiologic changes in two areas: (a) application of adverse event criteria (e.g., new guidelines regarding late effects, surgical and pediatric effects, and issues relevant to the impact of multi modal therapies); and (b) reporting of the duration of an effect. This instrument carries the potential to facilitate the standardized reporting of adverse events and a comparison of outcomes between trials and institutions (Trotti 2002).

Tools for grading late effects of cancer treatment are available for validation in larger populations and to examine their utility in survivors of adult cancers. Oncologists, primary care physicians, and ancillary providers should be educated and trained to effectively monitor, evaluate, and optimize the health and well being of a patient who has been treated for cancer. Additional research is needed to provide adequate knowledge about symptoms that persist following cancer treatment or those that arise as late effects especially among survivors diagnosed as adults. Prospective studies that collect data on late effects will provide much needed information regarding the temporal sequence and timing of symptoms related to cancer treatment. It may be clinically relevant to differentiate between onset of symptoms during treatment, immediately following treatment, and months to years later (Aziz 2002; Aziz and Rowland 2003). Continued, systematic follow up of cancer survivors will result in information about the full spectrum of damage caused by cytotoxic or radiation therapy and possible interventions that may mitigate these adverse effects.

The role of co-morbidities on the risk for, and development of, late effects of cancer treatment among, especially, adult cancer survivors has yet to be fully understood. Practice guidelines for follow-up care of cancer survivors and evaluation and management of late effects also have yet to be developed so that effects can be mitigated whenever possible (Aziz 2002; Aziz and Rowland 2003).

## 19.9 Advanced Illness

Care for patients with incurable disease has been recognized as an important component of quality care for cancer patients (Foley 2001). Patients with advanced illness face many of the same issues of other survivors, although issues may be magnified. The most common

problem for patients with advanced cancer is asthenia (Verger et al. 1992). There are many other specific pain and symptom management issues that present for patients with advanced illness that must be better understood so that improved treatments can be developed. Examples include malignant bowel obstruction (MBO), malignant ascites, fungating breast tumors, and painful bony metastasis. These are just some of the many problems that cancer patients frequently face with advanced disease. While there are many explanations as to why there is a paucity of data in many of these situations, it is an opportunity for researchers to explore best practices of care in these situations (Krouse et al. 2004). Research goals and standards must be equivalent to those for other medical specialties (Casarett 2002).

EOL care is a complex subject, and yet simply a focus on QOL of patients and their families is of utmost importance. There are many areas of need for patients and families, and resources are often available. However, EOL care in the U.S. is frequently substandard (2003). Patients too frequently die in the hospital; hospice services are either not available or not consulted until quite late in the patient's course, and too few palliative care specialists are available. The tardiness of hospice consultation may be related to multiple factors, including the poor prognostication of physicians (Lamont and Christakis 2001). Only a minority of hospitals have palliative care services to address the needs of patients and their families in the dying process (Billings and Pantilat 2001), and there are gaps in education of cancer specialists related to EOL care (McCahill et al. 2002; Cherny and Catane 2003). This is a specific area of needed focus for researchers and educators. Communication skills, knowledge of resources, planning for death, pain and symptom management, and advanced directives are all factors that must be considered in the optimal care for the patient facing death. There are multiple ethical dilemmas related to EOL care and research in this population, although opportunities are available to expand research in this population (Krouse et al. 2003, 2004). A recent meeting convened to augment research in this population focused on MBO as a model (Krouse 2007). This was chosen as it is a relatively common problem (Krouse et al. 2002; Fainsinger et al. 1994; Ripamonti and Mercadante 2004; Davis and Nouneh 2001) and has disparate treatment approaches that may be effective. Cultural, ethical, and implementation issues were noted to mandate special care in these trials (Fineberg et al. 2007; Laneader et al. 2007; Whalen et al. 2007). In addition, methods of measuring outcomes and benefit of treatments may differ from other types of trials (Mularski et al. 2007; Anthony et al. 2007). In addition, care should always be made to involve families in all decision-making processes, as they are frequently ignored or forgotten in the process of dying. The Education for Physicians on End-of-Life Care (EPEC) Project was developed by the American Medical Association (AMA) and funded by a grant from The Robert Wood Johnson Foundation. It is a series of courses concerning EOL issues. These courses are taught around the country, and materials can be obtained from the AMA website (http://www.ama-assn.org).

## 19.10 Future Directions

A large and growing community of cancer survivors is one of the major achievements of cancer research over the past three decades. Both length and quality of survival are important end points. Many cancer survivors are at risk for and develop physiologic and

psychosocial late and long-term effects of cancer treatment that may lead to premature mortality and morbidity. As in the past when treatments were modified to decrease the chance of toxicities in childhood cancer survivors, the goal of future research and treatment should also be to evaluate these adverse consequences systematically and further modify toxicities without diminishing cures. Interventions and treatments that can ameliorate or manage effectively both persistent and late medical or psychosocial effects of treatment should be developed and promoted for use in this population. Oncologists, primary care physicians and ancillary providers should be educated and trained to effectively monitor, evaluate and optimize the health and well-being of a patient who has been treated for cancer.

Additional research is required to provide adequate knowledge about symptoms that persist after cancer treatment or arise as late effects and interventions that are effective in preventing or controlling them. Continued, systematic follow-up of survivors will result in information about the full spectrum of damage caused by cytotoxic and radiation therapy and possible interventions that may mitigate the effects. Interventions, both therapeutic and lifestyle, that carry the potential to treat or ameliorate these late effects must be developed, and should be investigated in larger populations of cancer survivors, those with understudied cancer sites, and ethnocultural minority or medically underserved groups.

The relative lack of knowledge that currently exists about the physical health and quality-of-life outcomes of cancer survivors represents a clear area of challenge. It is also one for exciting opportunity and growth. Cancer is expected to become the leading cause of death in the future as a result of our aging population, reduced death rates from cardiovascular disease, and efficacious treatment and screening methodologies. Effective strategies to prevent and delay treatment-related physiologic and psychosocial sequelae must be developed, tested, and disseminated (if found to be effective) to achieve not only the goal of higher cancer cure rates but also a decreased risk of adverse health and social outcomes. As survivorship issues are increasingly explored and mandates are promoted from the survivor community, research in each of the areas described in this chapter should increase in the future (Tables 19.2 and 19.3). Ethical dilemmas and barriers to care and research must be addressed so that optimal follow-up and/or supportive care for cancer survivors across the trajectory of their experience post diagnosis continues to be studied and improved as patients live longer with the effects of cancer and its treatments.

**Table 19.2** Domains and priority areas for cancer survivorship research

| Survivorship research domain | Definition and potential research foci |
|---|---|
| Descriptive and analytic research | – Documenting for diverse cancer sites the prevalence and incidence of physiologic and psychosocial late effects, second cancers and their associated risk factors<br>• *Physiologic outcomes of interest* include late and long-term medical effects such as cardiac or endocrine dysfunction, premature menopause and the effect of other comorbidities on these adverse outcomes |

(continued)

**Table 19.2** (continued)

| Survivorship research domain | Definition and potential research foci |
|---|---|
| | • *Psychosocial outcomes of interest* include the longitudinal evaluation of survivors' QOL, coping and resilience, spiritual growth |
| Intervention research | – Examining strategies that can prevent or diminish adverse physiologic or psychosocial sequelae of cancer survivorship |
| | – Elucidating the impact of specific interventions (psychosocial, behavioral or medical) on subsequent health outcomes or health practices |
| Examination of survivorship sequelae for understudied cancer sites | – Examining the physiologic, psychosocial, and economic outcomes among survivors of colorectal, head and neck, hematologic, lung, or other understudied sites |
| Follow-up care and surveillance | – Examining the impact of high quality follow-up care on early detection or prevention of late effects |
| | – Elucidating whether the timely introduction of optimal treatment strategies can prevent or control late effects |
| | – Evaluating the effectiveness of follow-up care clinics/programs in preventing or ameliorating long-term effects of cancer and its treatment |
| | – Evaluating alternative models of follow-up care for cancer survivors |
| | – Developing a consistent, standardized model of service delivery for cancer related follow-up care across cancer centers and community oncology practices |
| | – Assessing the optimal quality, content and frequency, setting, and provider of follow-up care for survivors |
| Economic sequelae | – Examining the economic effect of cancer for the survivor and family and the health and quality-of-life outcomes resulting from diverse patterns of care and service delivery settings |
| Health disparities | – Elucidating similarities and differences in the survivorship experience across diverse ethnic groups |
| | – Examining the potential role of ethnicity in influencing the quality and length of survival from cancer |
| Family and caregiver issues | – Exploring the impact of cancer diagnosis in a loved one on the family and vice versa |
| Instrument development | – Developing Instruments capable of collecting valid data on survivorship outcomes and developed specifically for survivors beyond the acute cancer treatment period |
| | – Developing/testing tools to evaluate long-term survival outcomes; and those that (1) Are sensitive to change, (2) Include domains of relevance to long-term survivorship, (3) Will permit comparison of survivors to groups of individuals without a cancer history and/or with other chronic diseases over time |
| | – Identifying criteria or cut-off scores for qualifying a change in function as clinically significant (for example improvement or impairment) |

**Table 19.3** Future areas of research emphasis in long-term cancer survivorship research

| Area of research emphasis | Potential research questions |
|---|---|
| (A) Research related to specific survivor groups: | – What are the late or persistent effects of cancer and its treatment in *older* adult (65 years or older) long term cancer survivors? |
| (i) Those treated for previously understudied cancer sites (e.g., colorectal, gynecologic, hematologic, head and neck, lung) | – What is the health status, functioning, and QOL of long term cancer survivors belonging to diverse cancer sites? |
| (ii) Those belonging to understudied or underserved populations (adult, elderly, rural, low education/income, and diverse racial and ethnic populations) | – Which are the most common chronic and late effects among survivors across diverse cancer sites and which may be unique to subsets of different cancer survivor groups? |
| | – What are the characteristics of long-term survivors from rural communities and those from low income and educational backgrounds? |
| | – What are the similarities and differences in the survivorship experience among underserved cancer survivors and Caucasian survivors? |
| (B) Research addressing specific gaps in our knowledge: | (B) Research addressing specific gaps in our knowledge: |
| In particular as related to: | (i) Physiologic late or long-term effects |
| (i) Physiologic late or long-term effects | – Who is at risk for late and long-term effects and can they be protected? Are there specific, modifiable risk factors (other than exposure to treatment) for the development of late effects? |
| (ii) Psychosocial effects | – Which sub-groups of adult cancer survivors are at elevated risk for declines in functional status? |
| (iii) Interventions | – What are the most common late physiological sequelae of cancer and its treatment among adults, and their effect on physical and psychosocial health? |
| (iv) Health behaviors | – To what extent does cancer treatment accelerate age-related changes? |
| (v) Impact of cancer on family members | – Do co-morbidities affect risk for, development of, severity and timing of late effects of cancer treatment among adult cancer survivors? |
| (vi) Post treatment follow-up care, surveillance, and health care utilization | – What proportion of survivors will experience recurrent or second malignancies? |
| | (ii) Psychosocial effects |
| | – What are the psychosocial and behavioral consequences of late and or long-term physiological sequelae for survivors' health and well-being? |

(continued)

**Table 19.3** (continued)

| Area of research emphasis | Potential research questions |
|---|---|
| | – Which factors promote resilience and optimal well-being in survivors and their families? |
| | (iii) Interventions |
| | – Which interventions (medical, educational, psychosocial or behavioral) are most effective in preventing or controlling late or long term physiologic or psychosocial effects? When in the course of illness or recovery should they be delivered and by whom? |
| | – Can interventions delivered years after treatment control, reduce, or treat chronic or late cancer related morbidity? |
| | (iv) Health behaviors |
| | – Does regular physical activity after cancer (or avoidance of weight gain after hormonally dependent cancers) increase length and quality of survival? |
| | – Does having a cancer history alter cancer risk behaviors among long term survivors (e.g., smoking, alcohol consumption, sunscreen use)? |
| | (v) Impact of cancer on family members: |
| | – What long-term impact does cancer have on the functioning and well-being of family members of survivors? |
| | (vi) Post treatment follow-up care, surveillance, and health care utilization |
| | – Who is currently following cancer survivors for disease recurrence, and cancer treatment-related late and long-term effects? |
| | – What is the optimal frequency, content, and setting of post-treatment medical surveillance of cancer survivors, especially for those who are adults, and by whom should it be delivered? |
| | – How does cancer history affect subsequent health care utilization, both cancer-related and that associated with co-morbidities? |
| (C) Research that takes advantage of existing survivor cohorts or study populations | – Comparison of survivors' functioning over time and/or with other non-cancer populations (e.g., cohort or nested case-control studies) |

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